PB85-191559

Health Assessment Document for Dichloromethane (Methylene Chloride) Final Report

(U.S.) Environmental Protection Agency
Research Triangle Park, NC

Feb 85

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA/600/8-82/004F
February 1985
Final Report

Research and Development

PB85-191559

EPA

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NATIONAL TECHNICAL
INFORMATION SERVICE
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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA/600/8-82/004F

2.

3. RECIPIENT'S ACCESSION NO.
PB85 191559 /AS

4. TITLE AND SUBTITLE
Health Assessment Document for Dichloromethane
(Methylene Chloride)
Final Report

5. REPORT DATE
February 1985

6. PERFORMING ORGANIZATION CODE

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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
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Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

13. TYPE OF REPORT AND PERIOD COVERED
Final Report

14. SPONSORING AGENCY CODE
EPA/600/21

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16. ABSTRACT

Dichloromethane is used extensively in commercial and industrial solvent applications. The background atmospheric concentration is about 35 ppt. In surface water and drinking water, measured concentrations generally have been in the low parts per billion range. Available data suggest that DCM is biodegradable and has low aquatic toxicity to aquatic organisms.

The weight of evidence from the available literature indicates that adverse toxicologic (non-genotoxic) effects in humans are unlikely to occur at ambient air or water levels found or expected in the general environment. Available evidence suggests that the teratogenic potential of DCM for humans is minimal.

The weight of evidence with respect to mutagenic potential shows that DCM is capable of causing gene mutations and has the potential to cause such effects in exposed human cells. With regard to cancer, the weight of evidence from experimental animal studies is limited. When the absence of epidemiological evidence is considered with the limited animal evidence, the overall evaluation of DCM using the criteria of the International Agency for Research on Cancer is a Group 3 chemical in that it cannot be classified as to its carcinogenic potential for humans.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
Release to Public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
250

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/8-82/004F
February 1985
Final Report

Health Assessment Document For Dichloromethane (Methylene Chloride)

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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
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NOTICE

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PREFACE

The Office of Health and Environmental Assessment, in consultion with an Agency work group, has prepared this health assessment to serve as a "source document" for EPA use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards; however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed-effect levels and dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in some instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

In view of the pending release of a National Toxicology Program report on long-term animal studies with dichloromethane, the carcinogenicity conclusions of this document are considered interim and will be updated when the report can be evaluated.

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ABSTRACT

Dichloromethane (methylene chloride, DCM) is a volatile chlorinated hydrocarbon used extensively in commercial and industrial solvent applications. The background atmospheric concentration is about 35 ppt. In urban areas levels can be one to two orders of magnitude higher. In surface water and drinking water, measured concentrations generally have been in the low parts per billion range. Available data suggest that DCM is biodegradable and has low toxicity to aquatic organisms.

The weight of evidence from the available literature indicates that adverse toxicologic (non-genotoxic) effects in humans are unlikely to occur at ambient air or water levels found or expected in the general environment or even at higher levels sometimes observed in urban areas. Available evidence suggests that the teratogenic potential of DCM for humans is minimal.

The weight of evidence with respect to mutagenic potential shows that DCM is capable of causing gene mutations and has the potential to cause such effects in exposed human cells; additional studies are needed to determine the strength of evidence for mammalian systems. With regard to cancer, the weight of evidence from experimental animal studies is limited, according to the criteria of the International Agency for Research on Cancer. When the absence of epidemiological evidence is considered with the limited animal evidence, the overall evaluation of DCM, according to IARC criteria, is a Group 3 chemical in that it cannot be classified as to its carcinogenic potential for humans. Thus, additional testing would be warranted to clarify more adequately the carcinogenic potential of DCM for humans.

1. SUMMARY AND CONCLUSIONS

Dichloromethane (methylene chloride, DCM) is a solvent that is widely used for a variety of purposes. Annual production of DCM in the United States is about 269,000 metric tons. Approximately 85 percent of the DCM consumed in the United States is lost directly to the environment through dispersive use, largely by evaporation to the atmosphere. Natural sources have been proposed for DCM but none is believed to contribute significantly to ambient[a] concentrations. Although ambient air and water measurements are rather scarce, they indicate that DCM is found in a variety of urban and non-urban areas of the United States and in other regions of the world. The background atmospheric concentration is about 35 parts per trillion (ppt). Concentrations of DCM in urban areas could be one or two orders of magnitude higher than background. DCM is not expected to accumulate in the atmosphere; estimates of half-life vary from 20 days to 1 year. Hydroxyl radical attack is probably sufficiently rapid to prevent most, if not all, DCM from reaching the stratosphere.

DCM has been detected in both natural (fresh and seawater) and municipal waters in various geographic areas of the United States. Concentrations in the low parts-per-billion range have been measured in surface water and drinking water. DCM does not appear to be formed to any large extent during the chlorination process. DCM's short evaporation half-life from moving water probably allows most of the compound dissolved in water to be transported into the atmosphere. DCM also is readily degraded by bacteria in concentrations up to 400 parts per million (ppm). The extent to which DCM enters groundwater from surface waters is unknown. Some DCM is deposited in landfills. Where leaching is possible, the compound may enter groundwater systems because it does not easily adsorb to clay, limestone, and/or peat moss, and retention in the soil is unlikely. There is no evidence for significant bioaccumulation of DCM in the food chain. Data concerning the ecological consequences of DCM in the environment indicate that it is biodegradable under both aerobic and anaerobic conditions. DCM appears to have low toxicity to aquatic organisms.

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[a] Ambient is a term used in this and other documents of this nature to refer to the environment and should not be construed to include indoor and occupational settings.

As with other solvents of this class, inhalation of DCM in air followed by lung absorption is the most rapid route of entrance into the body. DCM also is well absorbed into the body after oral ingestion. Absorption through the intact skin occurs to some extent but is relatively a much slower process. Because of DCM's high solubility in water and lipids, it is probably distributed throughout all body fluids and tissues. DCM's half-time of elimination from adipose tissue (6 to 6.5 hours) is consistent with reports that DCM can be found in such tissue 24 hours after both single and chronic exposures. DCM readily crosses the blood-brain barrier, as evidenced by its narcotic effect at higher exposure levels. DCM also probably crosses the placenta and distributes into the developing fetus, but studies in experimental animals indicate that effects on the fetus are unlikely at levels commonly experienced by humans.

The absorption of ingested DCM is virtually complete. The amount of airborne DCM absorbed increases in direct proportion to its concentration in inspired air, the duration of exposure, and physical activity. At concentrations typical of ambient air, essentially all of the compound absorbed into the body is metabolized. DCM is known to be metabolized by the liver to carbon monoxide (CO). Carboxyhemoglobin (COHb) is formed from the interaction of CO and hemoglobin; CO dissociates at the lung and is eliminated. Blood normally contains about 0.5 percent COHb at all times; therefore, CO formed from a few ppt or ppb of DCM vapor is of no practical consequence. However, at higher exposure levels (500 ppm and above), the COHb level would be expected to reach a maximum between 12 and 15 percent in man. This level is below that considered hazardous for normally healthy individuals, but it could place additional stress on people with compromised cardiovascular systems or on pregnant women. The COHb formed from DCM metabolism is additive to COHb formed from exogenous CO. The results of animal experimentation by several investigators indicate that carbon dioxide (CO_2) is an additional metabolite of DCM. Thus, at least two pathways exist in rat liver for the metabolism of DCM.

The weight of evidence from the available literature indicates that adverse toxicologic (non-genotoxic) effects in humans are unlikely to occur at ambient air and water levels found or expected in the general environment or even at higher levels sometimes observed in urban areas. In fact, available experimental data do not indicate that any adverse toxicologic effects are induced in humans at the recommended Threshold Limit Value (TLV®) of 100 ppm (347 mg/m^3) DCM. The TLV® is a recommendation of the American Conference of Governmental Industrial Hygienists (ACGIH), 1983. The potential direct adverse health effect associated with exposure levels that greatly exceed 100 ppm (347 mg/m^3) is primarily neurological. The lowest concentration reported to affect eye-hand coordination was 200 ppm (695 mg/m^3).

Liver and kidney damage are unlikely to occur as a result of DCM exposure at environmental levels. Hepatotoxicity has not been reported in any human case report, even following fatal overexposure. Only minimal hepatic changes were observed in animal studies, even at doses ranging from the average LD_{50} (about 2 g/kg) to near-lethal levels. Animal studies also indicate that DCM has low nephrotoxicity.

Direct cardiac effects of DCM in humans also are unlikely because of the low levels of DCM found in the environment. Animal studies have shown that acute exposure levels exceeding 20,000 ppm (69,480 mg/m^3) are required before a decrease in myocardial contractility and other effects on cardiac performance are observed.

Available evidence suggests that the teratogenic potential of DCM in experimental animals is minimal. However, embryotoxicity, as manifested by lowered fetal weights and increases in skeletal variations, has been reported. These effects may be due to DCM metabolism. Since animal studies have been conducted using only very high doses, it is not possible to evaluate the dose-response relationship of DCM.

The weight of evidence with respect to mutagenic potential shows that DCM is capable of causing gene mutations and has the potential to cause such effects in exposed human cells. Positive responses to DCM exposure were observed in four different test systems. However, additional studies are needed to determine the strength of evidence for mammalian systems.

The evidence for the carcinogenic potential of DCM is based upon six chronic (lifetime) studies in which DCM was administered to rodent species. Four studies involved rats, one involved mice, and one involved hamsters. Chronic inhalation studies with rats and hamsters were conducted by the Dow Chemical Company. One rat study (Dow Chemical Company, 1980) showed a small increase in the number of benign mammary tumors compared to controls in female rats at all doses and in male rats at the highest dose, as well as a statistically significant increased incidence of ventral cervical sarcomas, probably of salivary gland origin in male rats. In hamsters, there was an increased incidence of lymphosarcoma in females only; this increase was not statistically significant after correction for survival. The results of the Dow Chemical Company (1980) study have since been published by Burek et al. (1984).

In the second inhalation study in rats by Dow Chemical Company (1982) there were no compound-related increased incidences of any tumor type, but the highest dose was appreciably lower than previously employed. A borderline hepatocellular adenoma and/or carcinoma response in female Fischer 344 rats and B6C3F1 male mice was observed only in the chronic drinking water study, which was conducted under the auspices of the National Coffee Association. However, while the response was significant with respect to matched controls, the incidence was within the range of historical control values at the performing laboratory.

The epidemiological data consist of two studies: Friedlander et al. (1978), updated by Hearne and Friedlander (1981); and Ott et al. (1983a, b, c, d, e). Although neither study showed excessive risk, both showed sufficient deficiencies to prevent them from being judged negative studies. The Friedlander et al. (1978) study lacked great enough exposure (based on animal cancer potency estimates) to provide sufficient statistical power to detect a potential carcinogenic effect. The Ott et al. (1983a, b, c, d, e) study, among other deficiencies, lacked a sufficient latency period for site-specific cancer.

Using the criteria of the International Agency for Research on Cancer (IARC), the weight of evidence for carcinogenicity in animals is judged to be limited. This conclusion is based upon the statistically positive salivary gland sarcoma response in male rats (Dow Chemical Company, 1980) and the borderline hepatocellular neoplastic nodule response in the rat and hepatocellular adenoma and/or carcinoma in male mice (National Coffee Association, 1982, 1983). The occurrence of limited carcinogenic activity is reason to suggest that additional testing is warranted to clarify adequately the carcinogenic potential. The National Toxicology Program (NTP) inhalation bioassay for DCM, due for release in early 1985, will clarify the issue. When the absence of epidemiological evidence is considered along with the limited animal evidence, as well as the potential for DCM to cause gene mutations in mammalian systems, DCM is judged to be in IARC Group 3 category. The IARC characterizes chemicals in this category as not being classifiable as to their human carcinogenicity.

While the qualitative classification for carcinogenicity is IARC Group 3, there is, nevertheless, a positive dose-response trend in the incidence of salivary gland sarcoma, and these data can be used to derive a rough estimate of potency for DCM. Such an estimate might be of use in a regulatory analysis of how severe a problem DCM exposure would be if it really were a carcinogen. The estimate is included here only for the purposes of a possible interim regulatory analysis pending the evaluation of the forthcoming NTP report. That report may clarify the nature of the carcinogenic response.

Note must be made of the aggregate findings for both mutagenicity and carcinogenicity. A positive finding of gene mutations is usually taken to support the likelihood that a chemical is a potential carcinogen. The mutagenicity findings, on the other hand, when considered in their own right, indicate a potential human adverse effect, although further studies are required to clarify the nature of the genetic damage in humans.

RECOMMENDATIONS FOR FURTHER STUDIES

Further research is needed in several areas. This list does not indicate research priorities.

(1) Reproductive Effects: It would be desirable to have more information on the dose-response relationship of DCM. Also, human epidemiology studies in women exposed to DCM would provide useful information on the potential for adverse pregnancy outcomes.

(2) Mutagenicity: Additional tests for chromosomal aberrations and aneuploidy should be conducted.

(3) Pharmacokinetics: There is a need to define more fully the pharmacokinetics so as to explain the carcinogenic responses or lack thereof and to clarify questions and hypotheses regarding mutagenicity.

2. INTRODUCTION

Dichloromethane is a high-volume industrial chemical widely used to remove paint and clean metal, and it is a solvent for aerosol sprays. This document provides an evaluation of the health effects of DCM and a review of the relevant available scientific literature. To provide a perspective in evaluating health effects associated with DCM, this document contains background chapters relating to analytical methodologies, production, sources and emissions, and ambient[a] air concentrations.

DCM is released into the ambient air as a result of evaporation during its production, storage, and manufacturing, or during general consumer use. It is believed to be derived from natural sources, but such formation is not believed to significantly contribute to global concentrations.

Information on the effects of DCM has been derived primarily from studies involving individuals occupationally or accidentally exposed to DCM. In such exposures, the concentrations of DCM associated with adverse effects to human health were either unknown or greatly exceeded the concentrations measured in ambient air. Controlled exposure studies have established that vapor inhalation is the principal route by which DCM enters the body. DCM is eliminated from the body primarily as the parent compound via the lungs. DCM is metabolized in animals and in humans to CO, which elevates the COHb content of blood. The distribution, storage, and metabolism of DCM to CO and CO_2 help explain its effects upon humans. Epidemiological studies provide some information about the impact of DCM on human health, but it is necessary to rely on animal studies to assess any indications of potentially harmful effects for chronic low-level exposures.

The primary concerns addressed in this document regarding the potential impact of DCM on human health are narcosis effects associated with acute high-level exposures and any mutagenic or carcinogenic effects potentially associated with chronic low-level exposures.

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[a] Ambient is used in this and other documents of this nature to refer to the environment and should not be construed to include indoor and occupational settings.

The U.S. Occupational Safety and Health Administration (OSHA) time weighted average health standard requires that a worker's exposure to DCM at no time exceed a time-weighted average (TWA)[a] of 500 ppm in the workplace air in any work shift of a 40-hr week. The TLV® for an 8-hr TWA concentration is 100 ppm with a 15-minute allowable excursion, for an average concentration of 500 ppm.

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[a] TWA is the concentration for a normal 8-hour workday and a 40-hour workweek to which nearly all workers may be exposed repeatedly, day after day, without adverse effect.

3. DICHLOROMETHANE: BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Dichloromethane is one member of a family of saturated aliphatic halogenated compounds. Other common names or synonyms are shown in Table 3-1. The Chemical Abstracts Service Registry Number for DCM is 000075092. Dichloromethane is a colorless, volatile liquid that is completely miscible with a variety of other solvents (Anthony, 1979). Although it has no measured flash point, it does possess flammable limits in air. The important physical properties of DCM are shown in Table 3-2. For example, DCM is highly volatile (vapor pressure of 350 torr at room temperature). Hence, the most common mode of entry into the body is inhalation. The ambient air concentration for compounds of this nature is often expressed in parts per million (ppm), parts per billion (ppb), and parts per trillion (ppt). At standard temperature and pressure, 1 ppm (v/v) is equivalent to 3.79 mg/m^3. In this document, a temperature of 25°C has been used. In all calculations, 1 ppm (v/v) is equivalent to 3.47 mg/m^3.

In the absence of moisture at ordinary temperatures, DCM is relatively stable compared with its congeners, chloroform and carbon tetrachloride. In dry air, DCM decomposition is initiated at temperatures above 120°C (Anthony, 1979). As moisture content increases, temperature decreases. The principal reaction product is HCl. Decomposition under these conditions can be inhibited by the addition of small quantities of phenolic compounds. On prolonged contact with water, DCM hydrolyzes very slowly, forming HCl as the primary product (Fells and Moelwyn-Hughes, 1958).

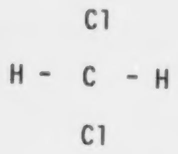
Although anhydrous DCM is noncorrosive to common metals, the HCl produced from hydrolysis of DCM initiates corrosive action with aluminum and iron (Anthony, 1979). The addition of epoxides to consume the HCl affords protection against this corrosion (Anthony, 1979). To minimize the decomposition of DCM, storage containers should be galvanized or lined with a phenolic coating (Anthony, 1979). Commercial grades of DCM contain a variety of stabilizers to minimize decomposition (McKetta and Cunningham, 1979). Cyclohexane, thymol, hydroquinone, p-cresol, and low-boiling amines have been used as stabilizers (De Forest, 1979). Aerosol preparations containing DCM often use propylene

TABLE 3-1. SYNONYMS AND IDENTIFIERS FOR DICHLOROMETHANE

Chemical Abstracts Service registry number: 000075092

Chemical formula: CH_2Cl_2

Structural formula:



$$\begin{array}{ccccc} & & Cl & & \\ H & - & C & - & H \\ & & Cl & & \end{array}$$

Synonyms:
Dichloromethane
Methylene dichloride
Methylene bichloride
Methylene chloride

oxide as a stabilizer. For preparations designed to be used in degreasing applications, special inhibitor mixtures are used. One such mixture includes propylene oxide, butylene oxide, cyclohexane, and N-methyl morpholine (De Forest, 1979).

The experimentally determined average evaporative half-life of DCM from water is 18 to 25 min (Dilling, 1977). In three separate experiments, Dilling used solutions of an average depth of 6.5 cm, containing approximately 1 ppm of DCM. The solutions were stirred at 200 revolutions per minute in a 250-ml beaker. These experimentally determined half-lives agreed with the value (20.7 minutes) obtained by the following formula:

$$t_{\frac{1}{2}} = \frac{0.06391d}{k_1} \qquad (3\text{-}1)$$

where d is the solution depth and k_1 is the liquid exchange constant (cm/min). The formula is an adaptation of the common equation for the half-life of a substance undergoing a first-order reaction. The calculated evaporative half-life may not be accurate for DCM in natural aquatic systems.

TABLE 3-2. SELECTED PROPERTIES OF DICHLOROMETHANE

Molecular formula	CH_2Cl_2
Formula weight	84.94
Boiling point (760 mm Hg)	40°C (760 mm Hg)
Melting point	-95 to -97°C
Vapor density	2.93 (air = 1)
Density of saturated vapor	2.06 (air = 1)
Density	1.326 g/ml (20°C)
Solubility	2.0 g/100 ml water at 20°C; soluble in ethanol, ethyl ether, acetone, and carbon disulfide
Explosive limits in oxygen	15.5 to 67% by volume
Flash point	None
Autoignition temperature	624° to 662°C
Relative evaporation rate	14 (water = 1) 71 (ether = 100)
Vapor pressure	Temp °F · Temp °C · mm Hg 50 · 10 · 230 68 · 20 · 349 77 · 25 · 436 86 · 30 · 511 95 · 35 · 600
Conversion factors (25°C; 760 mm Hg)	1 mg/l = 1 g/m^3 = 288 ppm 1 ppm (v/v) = 3.474 mg/m^3 = 3.474 μg/l
Concentration in saturated air	550,000 ppm (25°C)
Log octane/water partition	1.25

Sources: The above information has been compiled from a number of references including Hardie (1969), Anthony (1979), Dilling (1977), DeForest (1979), and American National Standards Institute (1970).

3.2 ENVIRONMENTAL FATE AND TRANSPORT

Because of its volatility and dispersive use pattern, much of the DCM produced worldwide is emitted into the atmosphere. Almost all of the emissions are from anthropogenic sources. DCM is also formed from natural sources, but natural sources are not believed to contribute significantly to global concentration (National Academy of Sciences, 1978).

3.2.1 Production

Dichloromethane is commercially produced in the United States, predominantly via the following reaction (De Forest, 1979; Anthony, 1979):

$$\begin{aligned} HCl + CH_3OH &\xrightarrow[130^\circ \rightarrow 180^\circ C]{FeCl_3 \text{ or } ZnCl} CH_3Cl + H_2O \\ CH_3Cl + Cl_2 &\xrightarrow{\hspace{3em}} CH_2Cl_2 + HCl. \end{aligned} \qquad (3\text{-}2)$$

In this vapor phase reaction sequence, yields of 95 percent are usual. Dimethyl ether is the secondary byproduct of the hydrochlorination.

A less common method used to obtain DCM is direct reaction of methane with chlorine at 485° to 510°C (Anthony, 1979). Methyl chloride, chloroform, carbon tetrachloride, and HCl are coproducts. However, both reactions are used in the chemical manufacturing industry so that the HCl can be recycled (Anthony, 1979).

In the liquid phase, DCM can be produced by refluxing and distilling a mixture containing methanol, HCl, and zinc chloride at 100° to 150°C. However, this method is not widely used (Anthony, 1979).

According to one source, production in the United States is carried out by five major companies at seven sites (Table 3-3).

According to statistics gathered by the U.S. International Trade Commission, the United States annual production of DCM was estimated to be 256,000 metric tons in 1980 (U.S. International Trade Commission, 1980) and 269,000 metric tons in 1981 (U.S. International Trade Commission, 1982).

Edwards et al. (1982) estimated 1981 world production at 825,000 metric tons.

TABLE 3-3. PRODUCERS OF DICHLOROMETHANE

Company	Production in Million Pounds Per Year
Diamond Shamrock	110
Dow Chemical	300
Stauffer Chemical	000[a]
LCP Chemical	52
Vulcan Materials	210

[a] Terminated Production

Source: Chemical Marketing Reporter, 1983.

3.2.2 Use

Dichloromethane is used as a paint remover, a urethane foam-blowing agent, a vapor degreasing and dip solvent for metal cleaning, a solvent for aerosol products, a solvent in the pharmaceutical industry, a solvent in the manufacture of polycarbonates by polymerization, and an extractant for caffeine, spices, and hops. DCM is used in the manufacture of plastics, textiles, photographic film, and photoresistant coatings, as a solvent carrier in the manufacture of herbicides and insecticides, and as a component of rapid-drying paints and adhesives, carbon removers, and brush cleaners. Other minor applications include use as a low-pressure refrigerant, a low-temperature heat transfer medium, and an air-conditioning coolant (Ahlstrom and Steele, 1979). Distribution of DCM by major use is shown in Table 3-4.

Dichloromethane is expected to retain popularity as a paint remover. Although DCM competes with trichloroethylene and perchloroethylene as a solvent, it is preferred as a paint remover because of its better performance (Lowenheim and Moran, 1975).

3.2.3 Emissions

Emissions from dispersive uses are the major source of DCM in the environment. Of the total amount of DCM produced in the United States, approximately 85 percent is estimated to be lost into the environment through sewage treatment

TABLE 3-4. CONSUMPTION OF DICHLOROMETHANE

Use	Percent of Production
Paint remover	25
Metal degreaser	10
Aerosol propellant	25
Blowing agent for foams	10
Export	12
Other	18

Source: Chemical Products Synopsis, 1981.

plants and surface waters, deposition on land, or loss to the atmosphere. The actual losses during production, transport, and storage are not well documented, but it appears that such losses represent only a very small percent of the DCM entering the environment from product manufacture and use. Most of the losses in production, transportation, and storage are fugitive (that is, transient) releases from leaky pump seals, valves, and joints. The dispersive uses of DCM are varied and widespread and are distributed geographically approximately with the industrialized population in the United States. Although most of the losses are to the atmosphere, DCM is relatively soluble in water. The most effective means of removal of DCM from water is air stripping, which transfers the chemical from water to the atmosphere (National Academy of Sciences, 1978).

3.2.4 Persistence of DCM

3.2.4.1 Atmospheric Degradation--Reaction with hydroxyl radicals (OH) is the principal process by which many organic chemicals, including DCM, are scavenged from the troposphere (Crutzen and Fishman, 1977; Singh, 1977; Altshuller, 1980). These radicals are produced by irradiating ozone (O_3), and the resultant singlet oxygen atoms then react with water vapor. The tropospheric lifetime of a compound can be related to the OH· concentration by the expression:

$$\tau_{\text{lifetime}} = \frac{1}{k\,[OH]} \qquad (3\text{-}3)$$

where k is the rate constant of reaction.

Available evidence indicates that the lifetime of DCM in the troposphere is less than 1 year. The modelling approaches of Crutzen and Fishman (1977) and Singh (1977) indicate that the range of the average OH concentration is between 2×10^5 and 6×10^5 molecules/cm^3. Using an average concentration of 3×10^5 molecules/cm^3, Altshuller (1980) calculated a tropospheric lifetime for DCM of 1.4 years. The rate constant expression of Davis et al. (1976) and a tropospheric temperature of 265°K were used.

Singh et al. (1979) computed a 1-year lifetime for DCM using the rate data reported by the National Aeroneutics and Space Administration (NASA, 1977) and the National Bureau of Standards (1978) and a temperature of 265°K. An average OH concentration of 4×10^5 molecules/cm^3 was employed in the computation. More recently, Singh et al. (1983) calculated an atmospheric residence time of 0.9 ± 0.3 year, based upon a comparison of atmospheric budget data with available emissions data. Since the mean concentrations in the northern hemisphere are 1.8 times the southern hemisphere values, a short lifetime for DCM is indicated.

However, Cox et al. (1976) calculated a 0.3-year lifetime in photokinetic studies in which DCM competed with nitrous acid as the target of OH attack. The lifetime was derived from a rate constant of 10.4×10^{-14} cm^3/molecule · sec at 298°K. An average OH concentration of 1×10^6 molecules/cm^3 was assumed. Use of 4×10^5 molecules/cm^3 for the OH concentration would have resulted in a calculated lifetime of 0.76 year; this value is closer to those of Singh (1977) and Altshuller (1980). Davis et al. (1976) calculated a lifetime of 0.39 year from a rate constant of 8.7×10^{14} at 265K and an average OH concentration of 9×10^5 molecules/cm^3.

Determination of the rate constant for the reaction of OH with DCM has been the focus of various investigations (Davis et al., 1976; Cox et al., 1976; Howard and Evenson, 1976; Perry et al., 1976). The values are in general agreement (Table 3-5) with the exception of Butler et al. (1978).

TABLE 3-5. REACTION RATE DATA FOR OH + CH_2Cl_2

($k \times 10^{-14} cm^3$/molecule · sec)	Temperature, K	Arrhenius expression	Reference
14.5 ± 2.0	298.5	---	Perry et al., 1976
15.5 ± 3.4	296	---	Howard and Evenson, 1976
11.6 ± 0.5	245 to 375	$4.27 \pm 0.63 \times 10^{-12}$ exp $[-(1094 \pm 81/T)]$	Davis et al., 1976
8.7	265	---	
10.4	298	---	Cox et al., 1976
2.7 ± 1	302	---	Butler et al., 1978
---	---	$5.2 \times 10^{-12} \exp(-1094/T)$	NASA,[a] 1977; National Bureau of Standards, 1978

[a] NASA preferred value; reliability of log k judged to be ± 0.2 at 230K.

3.2.4.2 Aquatic Biodegradation

--Recent evidence indicates that DCM is biodegradable under both aerobic and anaerobic conditions. Brunner and Leisinger (1978) first reported the isolation of a facultative methylotroph with the ability to utilize DCM as a sole carbon source for growth. The organism was tentatively identified as a *Pseudomonas* species. Brunner et al. (1980) observed that utilization of DCM is caused by an inducible enzyme, the activity of which is only partially inhibited under anaerobic conditions. Degradation of DCM by *Pseudomonas* sp., strain LP was also observed by LaPat-Polasko et al. (1984).

Rittman and McCarty (1980) investigated sewage microorganisms for their ability to biodegrade DCM. Bacterial cultures were enriched from a seed of primary sewage effluent over a 12-month period. DCM supported bacterial growth when the mineral salts culture medium was supplemented with sodium bicarbonate.

Klecka (1982) investigated the fate and effects of DCM in a system simulating a municipal waste water treatment plant. After acclimation, the sludge was used in closed-bottle respirometer studies. Respirometer studies demonstrated the disappearance of 10 mg DCM/l (^{14}C-labelled) within 4 hr, with 49 percent of the parent compound recovered as ^{14}C-labelled CO_2 after 50 hr. At 1 mg DCM/l, disappearance resulted within 3 hr, with a 66 percent conversion of the parent compound to CO_2 after 50 hr. Rate constants, calculated for the biodegradation of 1, 10, and 100 mg/l by activated sludge, were 1.42, 1.61, and 0.35/hr, respectively.

In inhibition studies on the effect of DCM on oxygen consumption and organic carbon degradation by activated sludge, Klecka (1982) found no significant effect over a 24-hr period. Modelling of a continuously mixed activated sludge reactor indicated that the rate of biodegradation was about 12 times greater than the rate of volatilization.

Wood et al. (1981) demonstrated the degradation of DCM under anaerobic conditions, using sediment-water samples spiked with 200 µg DCM. Degradation was observed to proceed via methyl chloride, although accumulation in the samples was not observed.

3.2.5 Products of DCM

3.2.5.1 Atmospheric Simulation Studies

--Dilling et al. (1976) observed that DCM was not very reactive in a chamber atmosphere containing nitric oxide (NO) or nitrogen dioxide (NO_2). Ozone-air mixtures containing 10 ppm (34.7 mg/m^3) DCM and 5 ppm NO or 16.8 ppm NO_2 were exposed to ultraviolet (UV) radiation at an intensity about 2.6 times that of natural sunlight at noon on a summer day in Freeport, Texas. After 21 hr of exposure in the presence of NO, less than 5 percent of the DCM had disappeared. Similarly, less than 5 percent disappeared in an NO_2 atmosphere after a 7.5-hr exposure. The effect of varying UV intensity or concentration on the rate of photodecomposition was not investigated. Relative humidity in the photolysis reactor was 35 to 40 percent. Further investigation of this simulated trospheric reaction showed anomalous behavior, possibly occurring on the chamber walls (Dilling and Goersch, 1980). Dichloromethane was judged to contribute to oxidant formation less than other halogenated compounds investigated, e.g., tetrachloroethylene, trichloroethylene, and vinyl chloride.

Butler et al. (1978) proposed that the ·OH attack on DCM in the presence of O_2 may result in formation of phosgene ($COCl_2$) via the following reaction sequence:

$$\begin{aligned} &CH_2Cl_2 + OH \rightarrow \cdot CHCl_2 + H_2O \\ &\cdot CHCl_2 + O_2 \rightarrow \cdot CHCl_2O_2 \\ &\cdot CHCl_2O_2 \rightarrow COCl_2 + OH. \end{aligned} \qquad (3\text{-}4)$$

This pathway was suggested to account for a low rate constant of the reaction of ·OH with DCM in the presence and absence of CO in the test atmosphere. Production of CO_2 was followed.

Chlorine-sensitized photooxidation of DCM in the presence of Cl_2 in dry air resulted in CO and CO_2 as the major carbon-containing products (Spence et al., 1976). After 5 min of irradiation of 20 ppm DCM and 5 ppm Cl_2 in air, 19 ppm CH_2Cl_2 was consumed. The product distribution was CO (5 ppm); HCl (38 ppm); $COCl_2$ (2 ppm); formyl chloride (0 ppm); and CO_2 (12 ppm). The product distribution is illustrative of a chain reaction:

$$\begin{aligned} &\cdot CHCl_2O_2 \rightarrow ClO + HCOCl \\ &HCOCl + Cl \rightarrow HCl + COCl \\ &COCl + O_2 \rightarrow CO_2 + ClO \\ &COCl \rightarrow CO + Cl \end{aligned} \qquad (3\text{-}5)$$

Chlorine-sensitized photooxidation of DCM is not expected to be significant under real atmospheric conditions because Cl will react with species other than halocarbons (Spence et al., 1976).

The hydrolysis of DCM in natural waters is expected to be influenced by temperature as well as by acidic and basic conditions. There is limited experimental evidence on the rate of hydrolysis of DCM under different conditions. Dilling et al. (1975) determined an experimental half-life of about 18 months (at 25°C). The pH was not stated. Fells and Moelwyn-Hughes (1958) reported a hydrolytic half-life of 13.75 days in acidic pollution at temperatures ranging from 80° to 150°C. When Dilling et al. (1975) extrapolated the Fells and Moelwyn-Hughes data to 25°C, the resultant half-life was about 680 years. Dilling et al. (1975) hypothesized that different mechanisms of hydrolysis may account for the discrepancy between experimental data at 25°C vs. extrapolated data from high temperature experiments.

Radding et al. (1977) in their literature survey reported a maximum hydrolytic half-life of 704 years (100° to 150°C at pH 7).

3.2.5.3 Sorption--Dilling et al. (1975) found that DCM could be adsorbed to dry bentonite clay and peat moss when these absorbents were added to a sealed solution containing DCM. However, the DCM that leaches from landfills adsorbs very little to clay, limestone, and/or peat moss, so retention in the soil is unlikely. These authors consider evaporation of DCM from water a more important process than adsorption.

3.3 LEVELS OF EXPOSURE

Dichloromethane has been detected in ambient air and in surface and drinking waters at numerous locations throughout the United States.

Dispersion models have been used to estimate population exposure to ambient DCM. The predicted maximum annual average to which people may be exposed is 14.3 ppb (0.050 mg/m^3), resulting from living near DCM production facilities. People living near other DCM sources such as organic solvent cleaning and paint and varnish removal operations are expected to be exposed to concentrations that do not exceed 7.1 to 14.3 ppb (0.025 to 0.050 mg/m^3) averaged over a year's time (Systems Applications, Inc., 1983).

Average background mixing ratios are approximately 30 to 50 ppt. In their review of ambient air data, Brodzinsky and Singh (1983) concluded that background levels are about 50 ppt, with many urban levels one or two orders of magnitude higher. Ambient air levels of DCM at various locations are shown in Table 3-6. Singh et al. (1983) reported that the average northern hemisphere background mixing ratio is approximately 38 ppt and the global average is 29 ppt. The Washington State University group reported free troposphere values between 30 and 40 ppt (Cronn et al., 1977; Robinson, 1978; Cronn and Robinson 1979; Grimsrud and Rasmussen, 1975; Rasmussen et al., 1979).

Pellizzari and Bunch (1979) have compiled a list of the sites in the United States at which Pellizzari and coworkers have identified DCM. DCM was sampled by adsorption onto Tenax® coupled with analysis by high resolution gas chromatography-mass spectrometry (GC-MS). The highest DCM concentration reported in New Jersey was at a waste disposal site in Edison. A level of 360 ppb was measured during an 11-minute sampling period in March 1976. During a 75-minute sampling period (November 1977), 55 ± 0.1 ppb DCM was detected at a site in Staten Island, New York. Longer sampling times (up to 8 hr) were required at sites in Virginia, West Virginia, and Pennsylvania to detect DCM. During a 7.25-hr period, about 70 ppb DCM was detected at a site in Front Royal, Virginia (October 1977). In the southwest, a level of about 1 ppb was reported at sites in Houston, Texas, during a 3-hr sampling period (October 1977). During considerably longer sampling periods (up to 24 hr), lower concentrations were reported for sites in Louisiana, e.g., Baton Rouge and Geismar. Sampling during a 48-hr period in Upland, California (August 1977) indicated concentrations of about 12 ± 9 ppb DCM.

3.3.1 Analytical Methodology

There are four practical methods to measure air concentrations of the halogenated hydrocarbons.

1. Gas chromatography with an electron-capture detector.
2. Gas chromatography-mass spectrometry.
3. Long-path infrared absorption spectroscopy, usually with preconcentration of whole air and then separation of the compounds by GC
4. Infrared solar spectroscopy, using the solar spectrum at large zenith angles to obtain greatest path lengths through the atmosphere.

TABLE 3-6. AMBIENT AIR LEVELS OF DICHLOROMETHANE

Location	Type of site	Date of measurement/ analytical method	Mixing ratio, ppb	Reference
Arizona				
Phoenix	Urban	April 23 - May 6, 1979/ GC-EC	Max 5.1552 Min 0.0859 Avg 0.8936 ± 0.9886	Singh et al., 1981
California				
Mill Valley	Background subject to urban transport	Jan. 11 - 27, 1977/ GC-EC	Max 0.087 Min 0.038 Avg 0.055 ± 0.014	Singh et al., 1979
Riverside	Urban	April 25 - May 4, 1977/ GC-EC	Max 0.473 Min 0.033 Avg 0.111 ± 0.094	Singh et al., 1979
Badger Pass	High altitude	May 5 - 13, 1977/ GC-EC	Max 0.126 Min 0.009 Avg 0.044 ± 0.025	Singh et al., 1979
Point Arena	Marine coastal	May 23 - 30, 1977/ GC-EC	Max 0.102 Min 0.013 Avg 0.045 ± 0.022	Singh et al., 1979
San Jose	Urban	Aug. 21 - 27, 1978/ GC-EC	Max 1.920 Min 0.060 Avg 0.401 ± 0.352	Singh et al., 1979
Point Arena	Marine coastal	Aug. 30 - Sept. 5, 1978/ GC-EC	Max 0.080 Min 0.016 Avg 0.039 ± 0.017	Singh et al., 1979
Los Angeles	Urban	April 9 - 21, 1979/ GC-EC	Max 12.0288 Min 0.6014 Avg 3.7511 ± 2.6203	Singh et al., 1981

(Continued)

TABLE 3-6. (continued)

Location	Type of site	Date of measurement/ analytical method	Mixing ratio, ppb	Reference
Oakland	Urban	June 28 - July 10, 1979/ GC-EC	Max 2.4058 Min 0.0859 Avg 0.4155 ± 0.3146	Singh et al., 1981
Upland*	Urban	Aug. 17 - Sept. 23, 1977/ GC-MS	Max 13 Min 0.54 Avg 8.5 ± 4.5	Pellizzari and Bunch, 1979
Kansas				
Jetmar	Remote	June 1 - 7, 1978/ GC-EC	Max 0.105 Min 0.033 Avg 0.054 ± 0.015	Singh et al., 1979
Louisiana[a]				
Baton Rouge	Urban	March 3 - May 20, 1977/ GC-MS	Max 0.55 Min 0.046 Avg 0.25 ± 0.17	Pellizzari et al., 1979
Geismar	Urban	March 1, 1977/ GC-MS	Max 0.33 Min 0.33 Avg 0.33 ± 0.062	Pellizzari, 1978a
Missouri[a]				
St. Louis	Urban	May 30 - June 8, 1980/ GC-EC	Max 0.62 Min 0.16 Avg 0.39 ± 0.14	Singh et al., 1980
Nevada				
Reese River	High altitude	May 14 - 20, 1977/ GC-EC	Max 0.099 Min 0.015 Avg 0.052 ± 0.022	Singh et al., 1979

(Continued)

TABLE 3-6. (continued)

Location	Type of site	Date of measurement/ analytical method	Mixing ratio, ppb	Reference
New Jersey[a]				
Bridgeport	Urban	Sept 22, 1977/ GC-MS	Max 0.26 Min 0 Avg 0.13 ± 0.19	Pellizzari and Bunch, 1979
Edison	Urban	March 24 - July 1, 1977/ GC-MS	Max 69 Min 0 Avg 26 ± 30	Pellizzari, 1978a; 1977
North Pacific Ocean				
37°N	Marine	April 1977/GC-MS	Avg 0.030 ± 0.008	Cronn et al., 1977
0°N - 33°N	Marine	Oct. 1976/GC-MS	Avg 0.033 ± 0.046	Robinson, 1978
Panama				
Canal Zone		July 1977/GC-MS	Max - Min - Avg 0.034	Cronn and Robinson, 1979
Southern Hemisphere				
0° to 42°S	Marine	Oct. 1976/GC-MS	Avg 0.035 ± 0.003	Robinson, 1978
77° to 90°S	Remote	Jan. 1977/GC-MS	Avg 0.034 ± 0.004	
32° to 55°S	Marine	Oct. 1977/GC-MS	Avg 0.040 ± 0.002	
77° to 90°S	Remote	Nov. 1977/GC-MS	Avg 0.033 ± 0.001	

(Continued)

TABLE 3-6. (continued)

Location	Type of site	Date of measurement/ analytical method	Mixing ratio, ppb	Reference
Texas				
Aldine*	Suburban	June 22 - Oct. 20, 1977/ GC-MS	Max 1.30 Min 0.29 Avg 0.84 ± 0.50	Pellizzari et al., 1979
Houston*	Urban	June 28, 1977, - May 24, 1970/GC-MS, GC-EC	Max 1.30 Min 0 Avg 0.57 ± 0.32	Pellizzari et al., 1979 Singh et al., 1980
Virginia*				
Front Royal	Urban	Sept. 29 - Nov 16, 1977/ GC-MS	Max 21 Min 0.49 Avg 8.5 ± 4.5	Pellizzari, 1978b
Washington				
Pullman	Rural	Dec. 1974 - Feb. 1975/ GC-MS	Max - Min - Avg <0.005	Grimsrud and Rasmussen, 1975
Pullman	Rural	Nov. 1975/GC-MS	Avg 0.035	Rasmussen et al., 1979

[a] GC-EC = gas chromatography-electron capture.
GC-MS = gas chromatography-mass spectrometry.

*Data obtained from summary report of Brodzinsky and Singh, 1983.

Each method has advantages and disadvantages and applications for which it is best suited. A major drawback of these techniques is that they do not allow real-time continuous measurements of the halocarbons at ambient levels in the environment.

The two most widely used systems for identifying and measuring trace amounts of DCM that occur in ambient air are GC-MS and gas chromatography-electron capture detection (GC-EC). Both systems have a limit of detection below 30 ppt. The GC-EC method has been reviewed by Pellizzari (1974) and by Lovelock (1974). The electron-capture detector (ECD) is specific in that halogenated hydrocarbons are quantitated, while non-halogenated hydrocarbons do not respond. Thus, high background levels of non-halogenated hydrocarbons in ambient air or water samples do not interfere with measurements of halogenated hydrocarbons. In a complex mixture in which several compounds may have similar retention times, alteration of the operating parameters of the GC-EC system will usually provide separation of the components. Linearity over a wide concentration range is achieved when the electron capture detector is used in the constant current mode. In this mode, the change in pulse frequency is linearly related to sample concentration. Nitrogen or a 95-percent argon/5 percent methane mixture is commonly used as the carrier gas.

3.3.2 Sampling of Ambient Air and Water

Contamination, absorption, and adsorption are common problems of the methods used to analyze air and water for DCM content. Four general approaches are used to collect samples of air for analysis of trace gas concentration: (1) cryogenic sampling in which liquid helium or liquid nitrogen is used to cool a container to extremely low temperatures; (2) pump-pressured samples, in which a mechanical pump is used without cryogenic assistance to fill a sampler to a positive pressure relative to the surrounding atmosphere; (3) ambient or subambient pressure sampling in which an evacuated container is simply opened and allowed to fill until it has reached ambient pressure at the sampling location; and (4) adsorption of selected gases on adsorbants such as molecular sieves or activated charcoal. Contamination and other problems are more serious in low-pressure sampling than in high-pressure sampling systems (National Academy of Sciences, 1978).

Water samples are subject to the same possibility of contamination and other problems that exist in air sampling. DCM aqueous samples must be carefully sampled, transported, and stored because of DCM's volatility and the complexity of the samples, especially those containing chlorine or other oxidants. A technique that is often used involves filling and sealing a serum bottle without air space and storing it just above freezing (Kopfler et al., 1976). Water samples generally require additional preparation before analysis. Normally, such samples may be concentrated by various water analysis techniques, but direct aqueous injection is used occasionally in GC analysis.

3.3.2.1 Sampling and Detection in Ambient Air--Several common approaches are used to sample ambient air for trace gas analysis, including the following approaches (National Academy of Sciences, 1978).

1. Pump-pressure samples. A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.

2. Ambient pressure samples: An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If filling is conducted at high altitude, the sample may become contaminated upon return to ground level.
3. Adsorption on molecular sieves, activated charcoal, or other sorbents.
4. Cryogenic samples: Air is pumped into a container and liquefied, and the partial vacuum that is created allows more air to enter. This method allows collection of several thousand liters of air.

Singh et al. (1979) have satisfactorily measured ambient levels of DCM by analysis with GC-EC. Samples were pressurized in stainless steel vessels, then preconcentrated by freezeout on 100/120 mesh glass beads (use of Tenax® monomer was discontinued because oxygen oxidized the monomer and interfered with EC). Separation was performed on a column containing 0.2 percent Chromosorb W 1500 80/100 mesh on Carbopack C. A post-column Ascarite water trap was used to remove water before EC. Dual detectors were used to provide a coulometric response.

Harsch et al. (1979) used GC-EC to identify and measure the level of DCM in samples of ambient air. A 500-ml sample was preconcentrated using the freezeout concentration method (Rasmussen et al., 1979). Halocarbons were desorbed onto a stainless steel column packed with 10 percent SF-96 on 100/120 mesh Chromosorb W. The reported detection limit was 26 ppt. Good separation from chlorofluorocarbon 113 (trichlorotrifluoroethane) was reported.

Cronn and Harsch (1979) reported a GC-MS detection limit of 6 ppt for a 500-ml aliquot of DCM. Cronn et al. (1977) also reported a detection limit of 20 ppt for a 100-ml aliquot. Pressurized air samples were separated on a column of Durapack n-octane on 100/120 mesh Porasil C. Cronn et al. (1976) have compared GC-MS with GC-EC in terms of precision and sensitivity. In general, GC-MS offered great specificity but could not equal GC-EC in reproducibility for the 11 halocarbons studied. For MS the detection limit for DCM was 9 ppt with a percent standard deviation of 13. The detection limit with temperature-programmed GC-EC was 4 ppt and the percent standard deviation was 7.3.

Pellizzari and Bunch (1979) reported an estimated detection limit of 200 ppt using a high-resolution GC-MS system in which DCM was first adsorbed onto a Tenax® GC. The accuracy of analysis was reported as ±30 percent. The inherent analytical errors are a function of several factors, including the following: (1) the ability to accurately determine the breakthrough volume; (2) the accurate measurement of the ambient air volume sampled; (3) the percent recovery of DCM from the sampling cartridge after a period of storage; and (4) the reproducibility of thermal desorption from the Tenax® cartridge and its introduction into the analytical system. Oxidation of the Tenax® monomer was not reported. Singh et al. (1982) have cautioned that the adsorption of halogenated compounds on Tenax® may not reliably reflect ambient air levels because measurements by some investigators have been reported as less than background (100 to 200 ppt).

Difficulties in using a coulometric approach to the GC-EC quantification of DCM in air samples have been reported by Lillian and Singh (1974). These investigators were unable to accurately measure DCM with detectors in series because of a greater-than-coulometric response. Dichloromethane was reported to have a very low ionization efficiency. The observed response might be attributed to the products of ionization having greater electron affinities than the reactants.

Cox et al. (1976) reported that polyglycol stationary-phase chemically bonded to porous glass (Durasil Low K1) was the only material found to separate DCM from other halocarbons during a GC-EC analysis. Satisfactory separation and analysis of DCM by GC-MS was reported by Grimsrud and Rasmussen (1975) with a 50-ft SCOT OV-101 column.

When the freezeout concentration method of Rasmussen et al. (1979) was applied to a 500-ml aliquot of air, the detection limit for DCM with GC-EC analysis was 4 ppt and the percent standard deviation was 26.2. The GC column contained 10 percent SF-96 on 100/120 mesh Chromosorb W.

The National Institute for Occupational Safety and Health (NIOSH, 1974) method P & CAM 127 is recommended for measurement of DCM in samples in which the concentration is greater than 0.05 mg DCM per sample. This method uses adsorption on charcoal followed by desorption with carbon disulfide. Analysis is made by GC with flame ionization detection. The mean relative standard deviation of the method is 8 percent. Otson et al. (1983) reported that this method is suitable, under certain conditions, for the simultaneous and quantitative determination of DCM and seven other chemicals, at and below their respective 8-hr TLV® values.

Grimsrud and Miller (1979) have reported an improved GC-EC method in which the detector response to DCM is enhanced by the addition of O_2 in the carrier gas. At the highest O_2 doping (5 ppth) the response of the detector to DCM (960 ppb) was enhanced 57-fold. The enhancement is depicted in Figure 3-1. A constant-current electron-capture detector was used.

3.3.2.2 Sampling and Detection in Water--A gas purging and trapping method suitable for DCM has been described by Bellar et al. (1979). Water samples are purged by bubbling with helium or nitrogen at 23°C. The halocarbons are adsorbed onto a porous polymer trap as the gas is vented. Quantification is made by GC-MS. Tenax® GC (60/80 mesh) was considered an effective adsorbent for compounds that boil above approximately 30°C. A recommended general purpose column is an 8-ft by 0.1-in. inside diameter stainless steel or glass tube packed with 0.2 percent Carbowax® 1500 on Carbopack® - C (80/100 mesh). For a sample volume of 5 ml, the range of the limit of detection is 0.1 to 1.0 µg/l.

3.3.2.2.1 Sample preservation (water). Bellar et al. (1979) recommended that water samples be stored in narrow mouth glass vials. Vials are filled to zero head space and covered with a Teflon®-faced silicone rubber septum. Screw caps are suitable seals. The presence of chlorine in water samples results in an increase in the concentration of certain halomethanes (not including DCM) upon storage.

3.3.2.2.2 Soil and sediment (water). Dichloromethane has been found in drinking and surface water at a variety of locations in the United States. In

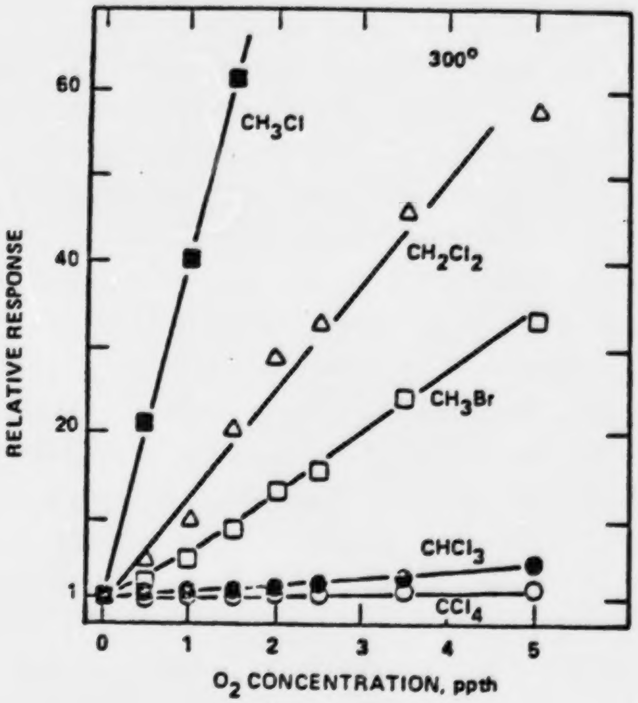


Figure 3-1. The effect of oxygen doping of the carrier gas on the ECD response to several halogenated methanes at a detector temperature of 300°C.

Source: Grimsrud and Miller, 1979.

a recent National Academy of Sciences report (1977), DCM formation resulting from chlorination treatment of water was reported.

In a survey for volatile organics in five drinking water supplies, Coleman et al. (1976) found that DCM was common to all the cities evaluated (i.e., Cincinnati, Miami, Philadelphia, and Ottumwa, Iowa). Concentrations were not reported. Analysis was performed using GC-MS.

In a 1975 survey by the U.S. Environmental Protection Agency (EPA) (1975a), DCM was detected in 9 of 10 water supplies. Lawrence, Massachusetts, had the highest concentration (1.6 μg/l). A mean concentration of less than 1 μg/l in finished water was reported in a survey of Region V water supplies (U.S. Environmental Protection Agency, 1975b). This survey indicated 8 percent of the finished-water supplies contained detectable DCM.

Dichloromethane was not among the major halogenated hydrocarbons detected by Dowty et al. (1975a) in New Orleans drinking water. In another report, DCM was detected in finished waters in the New Orleans area by GC-MS (Dowty et al., 1975a), and it was also found in Mississippi river clarifier effluent. The chlorocarbon was sorbed onto poly p-2,6-diphenyl phenylene oxide (35/60 mesh). Vapors were desorbed onto a capillary chromatographic column and quantitated by MS. Raw water influent was purified after clarifier treatment (sedimentation and some chemical treatment) to an extent that DCM concentration dropped 32 percent. However, the concentration in finished water increased after chlorination. Dichloromethane was also detected in commercially bottled artesian well water.

A slight increase in DCM concentration in chlorinated finished water was observed by Bellar et al. (1974). Finished water had 2.0 μg/l DCM after a complete treatment process of raw river water containing no detectable DCM. Water samples were also collected from various locations in a sewage treatment plant. Before treatment, the water contained 2.36 ppb DCM (8.2 μg/l). Before chlorination and after preliminary treatment, the water contained 0.8 ppb DCM (2.9 μg/l). After chlorination, the effluent contained 1 ppb DCM (3.4 μg/l). These tests show that DCM and other chlorocarbons may have formed as a result of the chlorination treatment. The most notable chlorocarbon was chloroform, for which an increase of 7.1 μg/l to 12.1 μg/l was observed after chlorination. GC-MS analysis was performed using a headspace preconcentration technique.

Dichloromethane was detected at 32 of 204 surface water sites from which samples were collected (Ewing et al., 1977). Sites were located near heavily industrialized river basins across the United States. Concentrations were reported as greater than 1 ppb (1 μg/l). Samples were collected from July 1975 to December 1976. Of the 204 sites, 91 were near major rivers and 57 were in tidal areas and estuaries. Samples (125 ml) were held at 60°C and stripped. Volatiles were sorbed onto Tenax® GC and desorbed onto Carbowax 1500 columns and analyzed by MS.

Dichloromethane was not among the contaminants detected by Sheldon and Hites (1978) in raw Delaware River water collected from August 1976 to March 1977.

Pellizzari and Bunch (1979) reported DCM in untreated Mississippi River water (Jefferson Parish, Louisiana) at a mean concentration of 2.581 μg/l. The highest value reported was 15.8 μg/l. Determinations were made from February 7 to August 5, 1977. A mean concentration of 0.13 μg/l was reported by Pellizzari and Bunch (1979) in tap water from Jefferson Parish. The highest level was 1.1 μg/l.

Data for DCM concentrations in general ambient waters in EPA's Storet files, covering the period January 1978 to April 15, 1981, indicate that levels ranged from 0 to 120 μg/l (U.S. Environmental Protection Agency, 1981). Sediment sampling detected DCM in 60 of 118 cases. Concentrations were from 427 to 433 ppb. Ambient soil concentrations of DCM are unknown (U.S. Environmental Protection Agency, 1981).

Singh et al. (1983) have detected DCM in seawater samples from the eastern Pacific Ocean. Mean surface concentrations of 2 ng/l were measured.

3.4 ECOLOGICAL EFFECTS

3.4.1 Effects on Aquatic Organisms

Dichloromethane has been tested for acute toxicity in at least seven aquatic species. The information in this section focuses upon observed DCM concentrations that were reported to result in adverse effects under laboratory conditions. Such parameters of toxicity are not easily extrapolated to environmental situations. Test populations may not be representative of the entire species because susceptibility to the test substance at different lifestages may vary considerably. Guidelines for the use of these data in the development of criteria levels for DCM in water are discussed in the Ambient Water Quality Criteria for Halomethanes (U.S. Environmental Protection Agency, 1980).

The toxicity of DCM to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods. The flow-through method continuously exposes the organism(s) to a constant concentration of DCM while oxygen is continuously replenished and waste products are removed. A static test exposes the organism(s) to the added initial concentration only. Results from both types of tests are commonly used as initial indications of the potential of substances to cause adverse effects.

3.4.1.1 Effects on Freshwater Species--Results of flow-through and acute static tests with DCM and freshwater species (fish and invertebrates) are shown in Table 3-7.

Alexander et al. (1978) used both flow-through and static methods to investigate the acute toxicity of several chlorinated solvents, including DCM, to adult fathead minnows (*Pimephales promelas*). Studies were conducted in accordance with EPA procedures described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Alexander et al. reported that the fish were observed for the following effects: loss of equilibrium, melanization, narcosis, and swollen, hemorrhaging gills. The DCM concentration that produced one or more of these observable effects in 50 percent of the fish (EC_{50}) was 99.0 mg/l for DCM. Fish affected during the exposure were transferred to static freshwater aquaria at the end of the 96-hour exposure period. Only the fish that were severely affected by high concentrations of the chemical did not recover. Short exposures to these compounds at the sublethal level seem to produce only reversible effects.

Using flow-through procedures, Black et al. (1982) determined the aquatic toxicity of DCM to embryo-larval stages of fish and amphibians. Exposure was initiated within 30 min of fertilization for fathead minnow (*Pimephales promelas*), rainbow trout (*Salmo gairdneri*), leopard frog (*Rana pipiens*), northwestern salamander (*Ambystoma gracile*), European common frog (*Rana temporaria*), and the African clawed frog (*Xenopus laevis*). Control and experimental eggs were simultaneously cultured under identical conditions. Sample size ranged from 50 to 125 eggs per exposure chamber. Evaporation of DCM was minimized by use of test chambers devoid of an air-water interface. Survival of normal rainbow trout larvae at 4 days posthatching was 100, 85, 44, and 9% at exposure levels of 0.008, 0.41, 23.1, and 36.5 mg/l, respectively. Teratic larvae were observed only at exposure concentrations of 5.55 mg/l or greater. The order of increasing species tolerance was *S. gairdneri*, *R. temporaria*, *A. gracile*, *P. promelas*, *X. laevis*, and *R. pipiens*. Respective

TABLE 3-7. EFFECTS OF DICHLOROMETHANE ON FRESHWATER SPECIES IN ACUTE TESTS

Species	Type of test	LC_{50} μg/l[a]	EC_{50}, μg/l[b]	Reference
Bluegill (*Lepomis macrochirus*)	static	224,000	-[c]	U.S. Environmental Protection Agency, 1980
Fathead minnow (*Pimephales promelas*)	static	310,000	-	Alexander et al., 1978
Fathead minnow (*Pimephales promelas*)	flow-through	193,000	-	Alexander et al., 1978
Daphnia magna	static	-	224,000	U.S. Environmental Protection Agency, 1980

[a] 96-hr test.

[b] 48-hr test.

[c] Data not available.

LC_{50} values were 13.1, 16.9, 17.8, ~34, >29, and >48 mg/l. LC_1 and LC_{10} values could only be calculated for R. temporaria. The respective values were 69.9 and 822.4 mg/l. The LC_1 and LC_{10} values suggested to the investigators that developmental stages of certain amphibian species may be affected by concentrations of 100 μg/l and that concentrations at or above 1 mg/l may produce substantial reproductive impairment.

Chronic test data concerning life cycle or embryo-larval tests are not available nor were data found on the chronic toxicity to invertebrates.

3.4.1.2 Effects on Saltwater Species--Static tests with mysid shrimp resulted in an LC_{50} value of 256,000 μg/l. No data exist on the chronic effects of DCM.

In a 96-hr static test with Sheepshead minnow (Cyprinodon variegatus) the LC_{50} value was 331,000 μg/l (U.S. Environmental Protection Agency, 1980).

3.4.2 Effects on Plants

The 96-hr EC_{50} values for DCM, based on chlorophyll a and cell numbers of the freshwater alga, Selenastrum capricornutum, were greater than the highest test concentration (662,000 μg/l). The 96-hr EC_{50} value based on chlorophyll a and cell numbers of the saltwater alga, Skeletonema costatum, was greater than the highest test concentration (662,000 μg/l) (U.S. Environmental Protection Agency, 1980).

Few studies of the effects on vascular plants are available (U.S. Environmental Protection Agency, 1981). Lehman and Paech (1972) tested the effect of DCM vapors on the photosynthetic fixation of $^{14}CO_2$ by alfalfa seedlings. At a very high concentration (21 percent), DCM reduced photosynthesis by 82 percent.

3.4.3 Bioconcentration Potential

Bioconcentration refers to increased concentration of a substance in the tissue of an organism (e.g., fish) relative to the ambient water concentration under steady-state conditions. A measure of the potential for organic chemicals to bioconcentrate in the fatty tissues of aquatic organisms is given by the octanol/water partition coefficient (Neely et al., 1974). The log octanol/water partition coefficient for DCM has been measured at 1.25 (Hansch et al., 1975), but no steady-state bioconcentration factor (BCF) has been measured. The BCF represents the ratio of the chemical concentration in the organism to that in the water. An approximate BCF for DCM of 5.2 has been calculated using the relationship Log BCF = 0.76 Log P - 0.23 (Veith et al., 1979). This estimated BCF places DCM in the low range of bioconcentration values, suggesting that the potential for bioconcentration in lipids is low. However, no information is available concerning DCM levels in living organisms (Pearson and McConnell, 1975) or the rate of transport of DCM through food chains.

However, the BCF alone may not be the most useful measure of the overall fate of a substance in water or its potential for producing toxic effects. Chemical and biological degradation of the substance, volatilization, desorption, and the depuration rate are among the key determinants of toxicity.

3.5 CRITERIA, REGULATIONS, AND STANDARDS

Permissible levels of DCM in the working environment have been established in various countries. The OSHA health standard requires that a worker's exposure to DCM at no time exceed 500 ppm (1,737 mg/m^3) TWA in any 8-hr work day of a 40-hr week, with an acceptable ceiling concentration of 1000 ppm (3,474 mg/m^3), that should not exceed 2000 ppm (6,948 mg/m^3) for more than 5 minutes in any 2-hr period. The American Conference of Governmental Industrial Hygienists (ACGIH) TLV® for inhalation exposure [200 ppm (695 mg/m^3)], proposed for prevention of narcotic effects and for protection against excessive COHb formation, has recently been lowered to 100 ppm (347 mg/m^3). The 8-hr TWA value in the Federal Republic of Germany is 100 ppm; in the German Democratic Republic and Czechoslovakia it is 144 ppm, and in Sweden it is 100 ppm. The acceptable ceiling concentration in the USSR is 14 ppm (49 mg/m^3). NIOSH has recommended that occupational exposure to DCM not exceed 75 ppm (261 mg/m^3), determined as a TWA for up to a 10-hr work day of a 40-hr week, in the absence of exposure to carbon monoxide above a TWA of 9 ppm for up to a 10-hr work day.

The Ambient Water Quality Criteria for Halomethanes (U.S. EPA, 1980) indicates that for the maximum protection of human health from the potential carcinogenic effects caused by exposure to any of several halomethanes (including DCM, chloromethane, bromomethane, bromodichloromethane, tribromomethane, dichlorodifluoromethane, and/or trichlorofluoromethane) through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the assumption of no threshold for these chemicals. However, zero level may not be attainable; therefore, the levels that may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria, based on data for chloroform, are 1.9, 0.19, and 0.019 μg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 157, 15.7, and 1.57 μg/l, respectively. Estimates for water consumption only were not made.

For halomethanes where one criterion is derived for an entire class of compounds, the U.S. EPA does not state that each chemical in the class is a carcinogen. The intended interpretation of the criterion is that the risk is less than 10^{-5} whenever the total concentration of all halomethanes in water is less than the criterion. In a hypothetical case where all of the halomethanes in a sample are non-carcinogenic, the criterion would be too strict. In most cases where halomethanes are detected, a mixture of compounds occurs and in calculation of the criterion, the assumption is made that all components have the same carcinogenic potency as chloroform.

The derived water quality criterion, based on non-carcinogenic risks and assuming a daily water intake of 2 l and consumption of 6.5 grams of fish and shellfish per day (bioconcentration factor 0.91) would be 12.4 mg/l.

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4. METABOLISM AND PHARMACOKINETICS OF DICHLOROMETHANE

Dichloromethane is a colorless liquid with a pleasant smell. The odor threshold has been reported to be about 100 ppm (347 mg/m^3) (May, 1966; Leonardos et al., 1969) and 250 ppm (868 mg/m^3) (Amoore and Hautala, 1983). Because of its relatively high vapor pressure at room temperatures (350 to 500 torr), DCM is readily absorbed into the body following inhalation, and most cases of severe poisoning from this solvent occur from inhalation exposures. Reports of poisoning in man by oral ingestion are rare (Llewellyn, 1966; Stewart and Hake, 1976; Friedlander et al., 1978), and total recovery has followed the swallowing of quite large doses (Roberts and Marshall, 1976).

Dichloromethane has been used as a general industrial solvent for at least six decades (Lehmann and Schmidt-Kehl, 1936), and its narcotic and anesthetic properties have been known to clinical medicine for over 60 years (Bourne and Stekle, 1923). However, most of the available information on DCM's metabolism and pharmacokinetics is derived from studies that were conducted within the last 10 years due to the resurgence of interest in DCM following the demonstration of its metabolism to CO (Stewart et al., 1972a,b). These studies in animals and humans have been carried out at relatively high oral or inhalation concentrations (50 to 1500 ppm) of DCM.

4.1 ABSORPTION, DISTRIBUTION, AND PULMONARY ELIMINATION

4.1.1 Oral Absorption

Absorption of DCM through the intestinal mucosa after ingestion appears to be rapid and complete. McKenna and Zempel (1981) obtained virtually complete recovery (92 to 96 percent) of ^{14}C-DCM radioactivity in urine, feces, and exhaled air of rats after single oral (gavage) doses of 1 or 50 mg/kg DCM in water. Pritchard and Angelo (1982) observed in B6C3F1 mice a peak blood concentration in less than 10 minutes following an oral gavage dose of 50 mg/kg DCM in either water or corn oil.

4.1.2 Dermal Absorption

Absorption of DCM through the skin from direct liquid contact or by the immersion of hands or arms is a slow process. Stewart and Dodd (1964) attempted to quantify the rate of absorption through human skin by immersing the thumbs of volunteers in liquid DCM and then determining the appearance and concentration of DCM in the breath. An estimate of the amount of DCM entering the body was made by comparison of these measurements with breath concentrations obtained following controlled inhalation exposures. From these breath analyses, Stewart and Dodd concluded that DCM is very slowly absorbed. They also suggested that although the amount of DCM absorbed depends on the area of the exposed skin, the slow rate of absorption would prevent toxic quantities of DCM from being taken into the body from direct contact with the skin of the hands and forearms. Immersion in DCM was found to be accompanied by an intense burning sensation within a few minutes.

4.1.3 Pulmonary Uptake

Inhaled DCM rapidly equilibrates across the alveolar endothelium because of its water and lipid solubility and the very large lung alveolar surface area. DCM is appreciably more water soluble (2 g/100 ml), but less lipid soluble than its congeners, chloroform and carbon tetrachloride; its lipid partition coefficient (olive oil/water) is 180 at 37°C with a blood/air coefficient of 7.9 (Lehmann and Schmidt-Kehl, 1936; Morgan et al., 1972; Lindqvist, 1978).

4.1.3.1 Studies in Humans--The magnitude of DCM uptake into the body (dose, burden) primarily depends on several parameters: inspired air concentration, pulmonary ventilation, duration of exposure, the rates of diffusion into blood and tissues, and solubility in blood and the various tissues. The concentration of DCM in alveolar air, in equilibrium with pulmonary venous blood content, asymptotically approaches the concentration in the inspiratory air until a steady-state condition is reached. After tissue and total body equilibrium is reached during exposure, uptake is balanced by elimination through the lungs and by other routes, including metabolism. The difference between alveolar and inspiratory air concentrations together with the ventilation rate (about 6 l/min at rest) provide a means of calculating uptake during exposure:

$$Q = (C_{insp} - C_{alv}) \dot{V} \cdot T \qquad (4\text{-}1)$$

where Q is the quantity absorbed, C is the concentration (inspired and alveolar) in mg/l, $\dot{V}$ is the alveolar ventilation rate in l/min, and T is the duration of exposure in minutes. The percent retention is defined as $(C_{insp} - C_{alv})/C_{insp} \times 100$, and percent retention x quantity inspired $(\dot{V} \cdot T \cdot C_{insp})$ is equal to uptake.

Figure 4-1 (from Riley et al., 1966) illustrates the overall time course of absorption and elimination during a 2-hour inhalation exposure of 100 ppm (347 mg/m^3) DCM for a 70-kg man. During exposure, the alveolar air concentration of DCM can be described by an exponentially rising curve with three components. At the beginning of exposure, an initial rapid rate of uptake occurs [0 to 50 ppm (0 to 174 mg/m^3) alveolar air], followed by a second slower uptake [50 to 65 ppm (174 to 226 mg/m^3) alveolar air]; finally, a very slow rate as equilibrium is approached at 70 ppm (243 mg/m^3) alveolar air concentration. The total quantity of DCM absorbed and retained in the body during exposure is represented by the area between the alveolar and inspired environmental air concentration curves. Complete equilibrium or steady-state conditions are not attained by the end of the 2-hour exposure to DCM, as shown by the slowly rising alveolar concentration curve. The three uptake compartments of the exponential alveolar curve correspond to equilibrium attained by first-order passive diffusion of DCM from blood. First, DCM diffuses through a vessel-rich group (VRG) of tissues with high blood flow (VRG: brain, heart, kidneys, liver, and endocrine and digestive systems), it then diffuses more slowly through the lean body mass (muscle group [MG]: muscle and skin) and last through adipose tissues (fat group [FG]). With the termination of exposure, blood and alveolar air DCM concentrations decline in parallel in an exponential manner with three components of pulmonary elimination and desaturation from VRG, MG, and FG body compartments. The VRG, MG, and FG groups are mathematically derived compartments used for convenience in analysis.

DiVincenzo and Kaplan (1981a) obtained serial breath excretion curves from four to six volunteers experimentally exposed to 50, 100, 150, and 200 ppm (174, 347, 521, and 695 mg/m^3) DCM for 7.5 hr under sedentary conditions. Pulmonary uptake was rapid during the first hour (exposure was interrupted at 4 hr, for 30 minutes). During the post-exposure period, DCM levels decreased rapidly (i.e., exponentially). Less than 0.1 ppm (0.347 mg/m^3) DCM was detected in the end tidal air of the individuals exposed to 50, 100, or 150 ppm (173, 347, or 521 mg/m^3) DCM 7 hr after exposure was terminated. For the 200-ppm (695 mg/m^3) exposures, the mean post-exposure end tidal air concentration of DCM decreased to 1 ppm (3.47 mg/m^3) at 16 hr. Post-exposure elimination of DCM was less than 5 percent of the amount absorbed. A related exposure

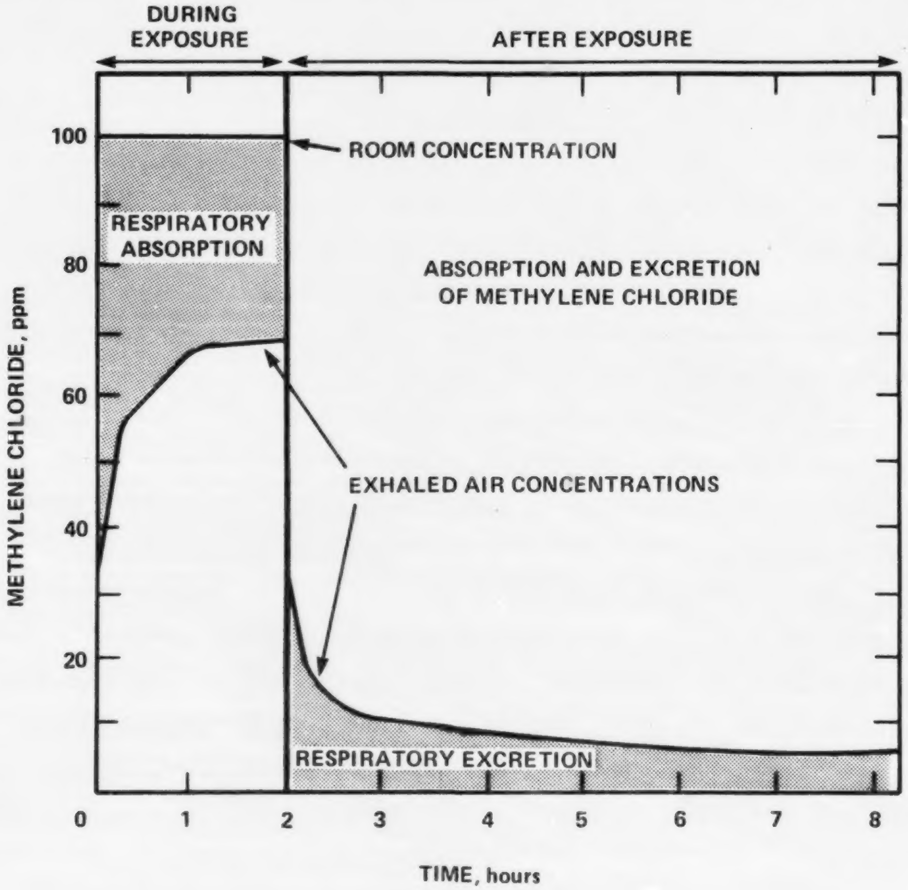

Figure 4-1. Inspired and expired air concentrations during a 2-hour, 100-ppm inhalation exposure to DCM for a 70-kg man, and the kinetics of the subsequent pulmonary excretion.

Source: Riley et al., 1966.

study in which volunteers were exposed to 100, 150, and 200 ppm (347, 521, and 695 mg/m^3) DCM for 7.5 hr daily for 5 consecutive days also indicated that uptake and elimination were directly proportional to the magnitude of exposure, thus confirming previous findings by DiVincenzo et al. (1972) and Stewart et al. (1973).

The retention of DCM as a percentage of inspired air concentration is independent of that concentration at equilibrium. Retention values for DCM, reported by different investigators, are shown in Table 4-1. These values have a large range and vary with the duration of exposure. Variation in the values is also caused by differences in body weights of the subjects and differences in body composition (proportion of adipose tissue to lean mass). For exposures greater than 1 hour, the mean retention approximates 42 percent of uptake of DCM or approximately 125 mg/hr for an exposure of 100 ppm (347 mg/m^3), assuming a resting ventilation rate of 6 l/min.

TABLE 4-1. PULMONARY ABSORPTION OF DCM BY HUMAN SUBJECTS UNDER SEDENTARY CONDITIONS

Investigator	Inhalation concentration, ppm	Exposure, hr	Retention, %
DiVincenzo and Kaplan, 1981a	50	7.5	70
	100	7.5	60
	150	7.5	63
	200	7.5	60
Lehmann and Schmidt-Kehl, 1936	662	0.3	74
	806	0.5	75
	1,152	0.5	72
	1,181	0.5	72
Riley et al., 1966	44-680	2	31
DiVincenzo et al., 1972	100	2	53
	100	4	41
	200	2	51
Astrand et al., 1975	250	0.5	55
	500	0.5	55
Engstrom and Bjurstrom, 1977	750	1	34

For short exposures, the quantity (dose) of DCM absorbed into the body is theoretically and experimentally directly proportional to the concentration of DCM in the expired air (Lehmann and Schmidt-Kehl, 1936; Astrand et al., 1975). The body burden of DCM also increases with exposure duration and with physical activity (increased ventilation and cardiac output) at a given inhaled air concentration (Engstrom and Bjurstrom, 1977; Astrand et al., 1975; DiVincenzo and Kaplan, 1981b). Astrand et al. (1975) found that physical activity during exposure to 250 and 500 ppm DCM (868 and 1735 mg/m^3) for 0.5 hour decreased retention from 55 percent in a resting stage to 40 percent during activity but that it doubled the amount of DCM absorbed because of a threefold increase of ventilation rate (6.9 to 22 ℓ/min). DiVincenzo and Kaplan (1981b) also found that physical exercise during exposure increased pulmonary uptake. Exposure of three males to 100 ppm DCM (347 mg/m^3) for 7.5 hours, during which they exercised on a treadmill for 5 minutes of each 15-minute period, also resulted in an estimated doubling of average cardiac output and as much as an eightfold increase in the average alveolar ventilation rate (Table 4-2). Effects upon blood COHb and conversion of DCM to CO are discussed elsewhere in this chapter.

One study attempted to correlate the quantity of DCM absorbed with body weight and fat content (Engstrom and Bjurstrom, 1977). Individuals were exposed to 750 ppm (2606 mg/m^3) DCM for 1 hour. Obese subjects (average body fat 25 percent of body weight) absorbed 30 percent more DCM than lean subjects (average body fat 8 percent of body weight). When fat biopsies of subcutaneous adipose tissue of obese subjects were taken, DCM concentrations of about 10 and 8 mg DCM/kg tissue weight were found at 1 and 4 hours post-exposure, respectively. Between 1 and 2 mg/kg were found in fat samples 22 hours after exposure. If confirmed, such observations would indicate that elimination of DCM from adipose deposits proceeds at a slow rate. Furthermore, the residual DCM in adipose tissue is additive to the next day's exposure, particularly in obese people. However, with multiple daily exposure, a total body equilibrium and constant adipose tissue concentration are eventually achieved with a given DCM concentration in inspired air.

4.1.3.2 Studies in Animals--McKenna et al. (1982) used 250-gram male Sprague-Dawley rats to estimate the total body burden of DCM resulting from 6-hour inhalation exposures of 50, 500, and 1500 ppm (173, 1737, and 5211 mg/m^3) of ^{14}C-DCM. After 6 hours, the animals were in apparent total body equilibrium with the inhaled concentrations of radioactive DCM, as suggested by "plateau"

TABLE 4-2. EFFECT OF EXERCISE ON PHYSIOLOGICAL PARAMETERS FOR VOLUNTEERS EXPOSED TO DCM

Volunteer	Work intensity[a] (ml O_2 min^{-1} kg b.w.$^{-1}$)	Aerobic capacity, %	Heart rate, beats/min	Estimated average cardiac output, l/min	Estimated average alveolar ventilation, l/min
1	4		56	5	8
	14	25	84	11	18
2	4		68	5	6
	15	25	96	12	34
	19	45	119	14	45
3	4		68	5	6
	16	45	123	15	28
	28	70	145	21	46

[a] Work intensity was estimated by monitoring the heart rate and relating it to equivalent O_2 consumption measured during pre-exposure aerobic capacity testing. Oxygen consumption at rest was taken as 4 ml O_2 min^{-1} kg body weight^{-1}, based on preexposure testing.

Source: DiVincenzo and Kaplan (1981b).

blood concentrations (Figure 4-2). The body burden was calculated from the total radioactivity recovered from exhaled air, urine and feces, and carcass analysis during the first 48 hours after exposure. Table 4-3 shows the body burden associated with each exposure. The increase in body burden of ^{14}C-activity was less than proportionate to the increments of DCM inhaled concentration.

4.1.3.3 Blood/Air Relationship--The blood concentration of DCM during inhalation and in the elimination phase after exposure parallels alveolar DCM concentration. This predictable relationship is defined by the solubility of DCM. Astrand et al. (1975) showed that for men exposed to 250 and 750 ppm DCM (868 and 2606 mg/m^3) for 1.5 hours, the arterial blood (mg/l) to alveolar air (mg/l) concentration ratios were constant and averaged 10.3 and 11.1, respectively, over threefold changes in alveolar concentrations. These *in vivo* Ostwald coefficients agree with the value found for blood/air (7.9) and for water/air (7.2) (Lindqvist, 1978; Morgan et al., 1972) at 37°C *in vitro*. The high water/air coefficient suggests that DCM is dissolved in plasma water as well as in lipid components of blood.

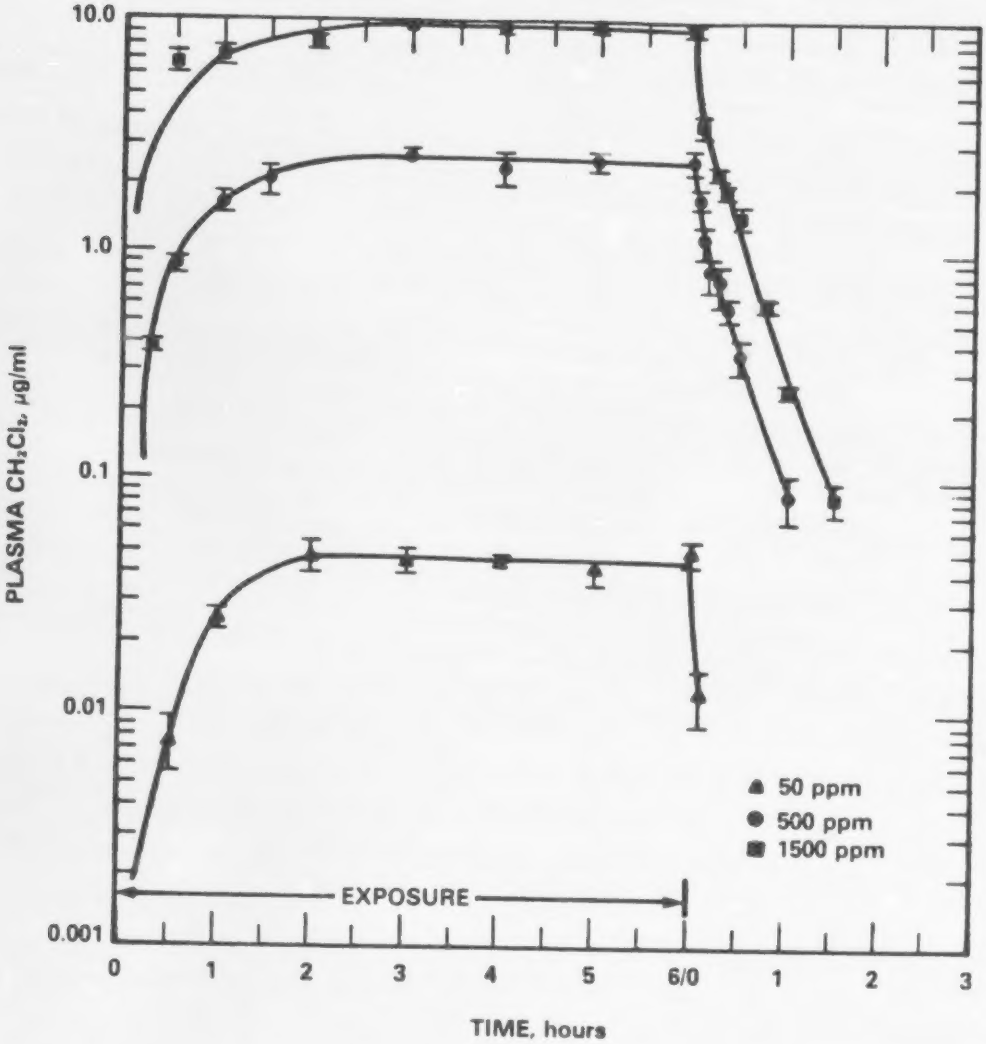

Figure 4-2. Plasma levels of DCM in rats during and after DCM exposure for 6 hours. Data points represent mean ± standard deviation for two to four rats.

Source: McKenna et al., 1982.

TABLE 4-3. BODY BURDENS OF RATS AFTER A 6-HOUR INHALATION EXPOSURE TO ^{14}C-DCM

Exposure concentration, ppm	Number of rats	Total body burden mg Eq ^{14}C-DCM/kg ± S.D.
50	3	5.53 ± 0.18
500	3	48.41 ± 4.33
1500	3	109.14 ± 3.15

S.D. = standard deviation.

Source: McKenna et al., 1982.

In the exposure study of DiVincenzo and Kaplan (1981a) described previously (Section 4.1.3.1), exposure and post-exposure blood concentrations of DCM were directly proportional to the magnitude of exposure. Blood/air coefficients calculated from the data suggest that the 200 ppm (694 mg/m^3) exposure concentration may be approaching the level at which saturation of metabolism occurs, as evidenced by an increased level of DCM in venous blood.

MacEwen et al. (1972) determined the blood DCM concentration in dogs that were continuously exposed for 16 days to 1000 and 5000 ppm DCM (3474 and 17,370 mg/m^3). The blood DCM concentrations were 36 and 183 mg/l, respectively, in direct proportion to exposure concentration. Total equilibrium can be assumed to have occurred in these animals. Ostwald coefficients of 10.4 and 10.5 for the two exposure concentrations agree with the above values noted in man. Similar values can be calculated from the data of Latham and Potvin (1976); Figure 4-3 shows the proportional relationship they found in rats between DCM blood and inspired air concentrations over a range of 1,000 to 8,000 ppm (3,474 to 27,792 mg/m^3) during a 6-hour exposure.

In contrast to these findings of a direct proportional relationship between inspired air concentration of DCM and blood level in man and other animals, McKenna et al. (1982) reported a greater than proportionate increase of blood concentration with inhalation exposure concentration in rats. Table 4-4 gives the apparent steady-state concentration of DCM in plasma and whole blood of Sprague-Dawley rats exposed for 6 hours each to 50, 500, and 1500 ppm DCM (173, 1737, and 5211 mg/m^3). The data indicate that the whole blood and

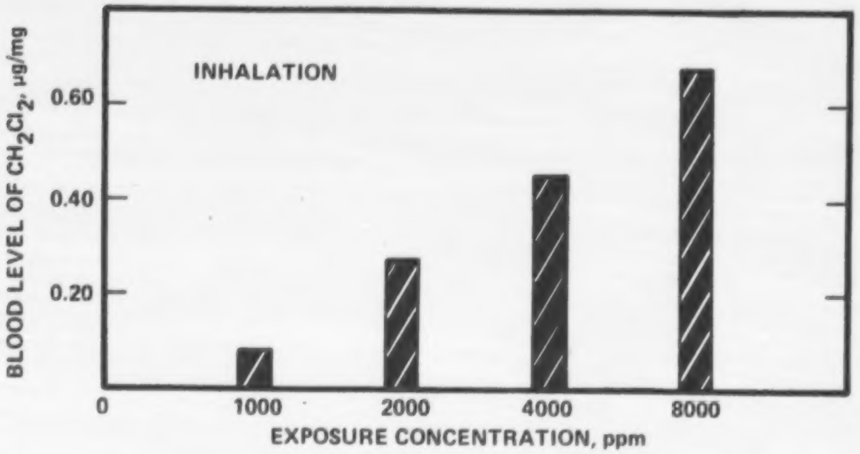

Figure 4-3. DCM venous blood levels in rats immediately after a single 6-hr inhalation exposure to various concentrations of DCM.

Source: Latham and Potvin, 1976.

TABLE 4-4. DCM CONCENTRATIONS IN RAT WHOLE BLOOD AND PLASMA AT APPARENT STEADY-STATE CONDITIONS AFTER A 6-HOUR INHALATION EXPOSURE

Exposure concentration, ppm	Number of rats	DCM concentration μg/ml ± S.D. Whole blood	DCM concentration μg/ml ± S.D. Plasma	Plasma/blood distribution coefficient
50	3	0.22 ± 0.04	0.05 ± 0.01	0.23
500	3	-	2.38 ± 0.42	-
1500	3	39.53 ± 3.71	8.94 ± 0.39	0.23

S.D. = standard deviation.

Source: McKenna et al., 1982.

plasma levels of DCM increase disproportionately with an increase in inspired air concentration. Furthermore, calculation of the blood/air ratio for these data provide increasing values of 5.75, 5.97, and 7.59 for 50, 500, and 1,500 ppm (173, 1737, and 5211 mg/m^3), respectively. McKenna et al. suggest that the resultant increase in blood DCM concentration is greater than that predicted by increments in the inspired air because of a rate-limited metabolism of DCM in the rat. Thus, at low inspired air concentrations (below saturation of metabolism), the blood/air ratio is less (because of rapid metabolism) than that at high inspired air concentrations (above saturation of metabolism);

indeed, the blood/air coefficient (7.59) observed at 1,500 ppm DCM (5,211 mg/m^3) agrees with coefficients observed by others.

4.1.4 Tissue Distribution

Because of DCM's water solubility, it probably distributes throughout the body water, and its lipid solubility allows its distribution into all body tissues and cellular lipids, particularly into adipose tissue. Engstrom and Bjurstrom (1977) determined that the Ostwald coefficient for subcutaneous adipose tissue from human buttocks is 51 at 60°C; this value indicates that the tissue/blood partition coefficient may be about 7 at body temperature (37°C).

DCM readily crosses the blood-brain barrier even at relatively low vapor exposure concentrations, as evidenced by its impairment of manual and mental performance at 500 ppm (1737 mg/m^3) (Winneke and Fodor, 1976; Winneke, 1981). DCM also crosses the placenta and may affect fetal development (Schwetz et al., 1975; Anders and Sunram, 1982). Schwetz et al. (1975) consider the delay in rat fetal development to be a result of maternal toxicity.

4.1.4.1 Animal Studies--Tissue concentrations of DCM increase with exposure concentrations and duration and for any given tissue are dependent on the largely unknown tissue partition coefficients. Savolainen et al. (1977) chronically exposed rats to DCM [200 ppm (695 mg/m^3), 6 hr daily, for 5 days] and determined DCM concentrations in perirenal fat and other tissues. The resulting data, shown in Table 4-5, indicate that significant amounts of DCM remained in perirenal fat 18 hr after the previous exposure of day 4, and increased markedly with a 6-hr exposure on day 5. At termination of the last (fifth daily) 6-hr exposure, the ratio of tissue to blood concentrations was about one for brain and liver and 6.6 for perirenal fat.

Previous work by DiVincenzo et al. (1972) in man and Carlsson and Hultengren (1975) in rats indicated that little uptake by adipose tissue occurs. However, single, short exposure periods of 2 hours were used in their studies, and of the smaller amount absorbed, 95 percent was probably accomodated in VRG and MG compartments because the FG compartment receives only 5 percent of the cardiac output.

McKenna and coworkers (1981, 1982) have studied DCM distribution in tissues of rats by observing ^{14}C-activity 48 hr after a single 6-hr inhalation of 50, 500, or 1500 ppm DCM (174, 1737, and 5211 mg/m^3) and after single oral

TABLE 4-5. TISSUE CONCENTRATIONS OF DCM IN RATS EXPOSED TO 200 ppm DCM FOR 4 DAYS FOR 6 HOURS DAILY

Exposure on the fifth day, hr	DCM concentrations nmol/g tissue wet weight ± S.D.				
	Cerebrum	Cerebellum	Blood	Liver	Perirenal fat
0	-	-	-	-	113 ± 29
2	73 ± 20	57 ± 20	90 ± 10	85 ± 2	526 ± 94
3	119 ± 33	86	79 ± 3	82 ± 1	537 ± 33
4	57 ± 8	95 ± 8	120 ± 10	101 ± 13	608 ± 58
6	83	90	100 ± 1	83 ± 10	659 ± 77
Tissue to blood coefficient after the fifth daily exposure					
	0.83:1	0.90:1	1.0:1	0.83:1	6.59:1

S.D. = standard deviation

Source: Savolainen et al., 1977.

doses of 1 or 50 mg/kg. The results are shown in Table 4-6. Following the inhalation exposures and the oral dosage, the highest concentrations of ^{14}C-activity were found in the liver, kidney, and lung. The observed ^{14}C-activity in epididymal fat was consistently lower than that observed in either whole blood or the remaining carcass. The tissue ^{14}C-activity increased with dose, but it did not increase in direct proportion to increasing dose. Intact DCM was not detected in any of the tissues assayed; therefore, the observed radioactivity is presumed to represent nonvolatile metabolites of DCM. These results contrast with those of Savolainen et al. (1977), who found that in perirenal adipose tissue, significant amounts of intake DCM remained 18 hr after the last of four daily 6-hr inhalation exposures to 200 ppm DCM (695 mg/m^3) (Table 4-5).

The results of the experiments of Savolainen et al. and McKenna et al. indicate that total body equilibrium of DCM to inspired air concentration is not achieved in the rat within a single 6-hour inhalation period even though this response is suggested by the achievement of a "plateau" blood concentration as shown in Figure 4-2. Also of interest is the evident difference between the amount of tissue ^{14}C-metabolites associated with inhalation and oral dosage. A close correspondence exists between tissue metabolites of DCM

TABLE 4-6. DISTRIBUTION OF ^{14}C-ACTIVITY IN RAT TISSUE 48 HOURS AFTER 6-HOUR INHALATION EXPOSURE OR ORAL DOSAGE OF ^{14}C-DCM

Exposure method	mgEq ^{14}C-Activity by exposure concentrations, tissue ± S.D.		
Inhalation	50 ppm	500 ppm	1500 ppm
Liver	8.4 ± 1.5	35.6 ± 7.5	44.2 ± 3.5
Kidney	3.3 ± 0.1	16.2 ± 2.4	30.5 ± 0.2
Lung	1.9 ± 0.2	11.0 ± 1.3	16.5 ± 1.6
Brain	0.8 ± 0.3	4.2 ± 1.3	6.7 ± 0.2
Epididymal fat	0.5 ± 0.2	6.5 ± 0.5	4.1 ± 0.9
Skeletal muscle	1.1 ± 0.1	4.4 ± 1.9	7.7 ± 0.7
Testes	1.1 ± 0.2	5.5 ± 1.3	8.1 ± 0.5
Whole blood	1.1 ± 0.2	8.1 ± 1.9	8.9 ± 1.7
Remaining carcass	1.3 ± 0.2	5.6 ± 0.9	8.6 ± 1.4
Oral	1 mg/kg	50 mg/kg	
Liver	0.40 ± 0.04	6.67 ± 0.69	
Kidney	0.15 ± 0.01	2.92 ± 0.21	
Lung	0.99 ± 0.01	1.67 ± 0.16	
Brain	0.04 ± 0.06	0.63 ± 0.10	
Epididymal fat	0.02 ± 0.002	0.33 ± 0.0006	
Skeletal muscle	0.08 ± 0.02	0.86 ± 0.04	
Testes	0.04 ± 0.002	0.92 ± 0.10	
Whole blood	0.06 ± 0.003	1.41 ± 0.11	
Remaining carcass	0.05 ± 0.002	0.99 ± 0.60	

Number of animals in each group = 3

Source: McKenna et al., 1982; McKenna and Zempel, 1981.

for an oral dose of 50 mg/kg and a 6-hr inhalation exposure of 50 ppm, although 50 ppm (174 mg/m^3) provides a terminal body burden of only 5.5 mg/kg (Table 4-3).

4.1.5 Pulmonary Elimination

Pulmonary excretion is the mechanism of elimination of virtually all unchanged DCM from the body. Less than 2 percent of estimated body doses of DCM have been detected as unchanged compound in the urine of human subjects exposed to 100 ppm and 200 ppm DCM (347 and 695 mg/m^3) for 2 hours (DiVincenzo et al., 1972) and in the urine of dogs exposed to 5000 ppm (17,370 mg/m^3) (MacEwen et al., 1972).

4.1.5.1 Studies in Humans--Figure 4-1 schematically shows the time-course of pulmonary elimination of DCM after inhalation exposure. The parameters of elimination equilibration of the body are the same as those of assimilation equilibration. After termination of exposure, DCM immediately begins to be eliminated from the body via the lungs. Alveolar air equilibrates with pulmonary venous blood whose DCM concentration becomes a function of the first-order diffusion of DCM from tissues, the arterial blood flow/tissue mass, and the relative solubilities of DCM in tissues. Figure 4-1 shows that alveolar DCM concentration follows an exponential decay curve with three major components reflecting desaturation of the VRG, MG, and FG compartments, respectively. The half-times of elimination of DCM from these compartments have not been firmly established. Riley et al. (1966) measured expired air concentration after termination of exposure and found half-times of 5 to 10 min for the VRG compartment, 50 to 60 min for the MG compartment, and 400 minutes for the FG compartment. DiVincenzo et al. (1972), who exposed subjects to 100 ppm and 200 ppm DCM (347 and 695 mg/m^3) for 2- and 4-hr periods, felt that "very little vapor" reached the fat stores and muscle tissues under these conditions. DiVincenzo et al. found DCM to have a half-time value in blood of 40 min following 2 hr of exposure; prolonging exposure to 4 hr had no significant effect on the half-time. DiVincenzo and Kaplan (1981a,b) have recently extended previous studies by following the course of pulmonary elimination in individuals exposed for 7.5 hr to 50, 100, 150, and 200 ppm DCM (174, 347, 521, and 695 mg/m^3) and in individuals exposed for 7.5 hr daily for 5 consecutive days to the above three highest concentrations. The authors concluded that post-exposure elimination of DCM in breath is a minor route of elimination over the concentration range used; post-exposure elimination was less than 5 percent of the amount absorbed. Pulmonary elimination of DCM was rapid at all exposure concentrations. Less than 0.1 ppm (0.347 mg/m^3) was detected in post-exposure breath samples at 7 hr. Die-away of DCM in blood paralleled that observed with pulmonary eliminations. Breath elimination times were prolonged by physical exercise. Morgan et al. (1972), using isotopically labeled DCM, estimated the half-time of DCM in the VRG compartment as 23 min. Engstrom and Bjurstrom (1977) reported the half-time of DCM in the MG compartment for lean subjects was about 60 min; these authors obtained a longer time value for obese subjects. For both lean and obese subjects, biopsies indicated that residual concentrations of DCM existed in adipose tissue nearly 24 hr after exposure, suggesting that the half-time of elimination from the FG compartment is fairly long. From the post-exposure alveolar concentration curves prepared by Stewart et al. (1976a) for subjects exposed to inhalation concentrations of 50 to 500 ppm DCM (174 to 1,737 mg/m^3) for 1 to 7.5 hr/day for 5 successive days, the half-time of elimination for MG and FG can be estimated as 60 to 80 min, and 240 min, respectively. The best guesses from these studies for the half-times of elimination from the VRG, MG, and FG are 8 to 23 min, 40 to 80 min, and 6 to 6.5 hr, respectively. The long half-time from elimination of the adipose tissue compartment and reports that DCM remains in this compartment 24 hr after single and chronic exposures indicate that the concentration of DCM in adipose tissue may only slowly, over a period of days, achieve equilibrium with inspired air concentration, particularly for daily exposures of short duration.

4.1.5.2 Studies in Animals--The estimates of half-times of pulmonary excretion of DCM in animals are derived from post-exposure alveolar concentration curves. Blood DCM decay concentration curves are a less reliable parameter of pulmonary excretion because these curves are influenced by metabolism, the other major route of elimination of DCM. McKenna et al. (1982) determined the pharmacokinetic half-times for elimination of DCM from plasma of rats following a single inhalation exposure to 500 or 1500 ppm DCM (1737 or 5211 mg/m^3) for 6 hours. Figure 4-2 shows that two first-order processes governed the disappearance of DCM from plasma with apparent half-times of 2 and 15 minutes for the rapid and slow components of elimination, respectively. The kinetic parameters calculated from these curves are summarized in Table 4-7.

The kinetic parameters for rats differ greatly from those of man. Presumably, the half-times for the fast and slow components correspond to the half-times for human VRG and MG compartments. The half-time for the FG or adipose tissue compartment was not established in the rat because plasma decay was only followed for 1.5 hours following inhalation exposure (Figure 4-2). The rates of elimination of DCM from plasma in the rat are first order and independent of dose, although there is considerable evidence of extensive metabolism in these animals (Section 4.2).

McKenna and Zempel (1981) have also determined the kinetics of pulmonary excretion of DCM in rats after a single oral dose (via gavage) of 1 or 50 mg ^{14}C-DCM per kg body weight. Figure 4-4 shows the time course (for 5 hours) of DCM in exhaled air of these rats. Pulmonary elimination of DCM from rats

TABLE 4-7. COMPARISON OF POST-EXPOSURE PULMONARY ELIMINATION HALF-TIMES OF DCM FOR HUMANS AND RATS

Subject	Exposure method	DCM concentration	$T_{1/2}$ first-order components (minutes)		
			Alpha	Beta	Gamma
Human	Inhalation	50-500 ppm	8-23	40-80	360-390
Rat	Inhalation	500 ppm (6 hr)	2.4	14.8	-
		1500 ppm (6 hr)	1.06	15.5	-
Rat	Gavage	1 mg/kg	-	12.6	46.5
		50 mg/kg	-	12.6	46.6

Source: McKenna et al., 1982.

receiving the 1 mg/kg dose is characterized by a two-component decay curve representing two first-order processes with half-times of elimination of 12.6 minutes and 45.6 minutes, respectively. The pulmonary excretion of DCM from rats given the 50 mg/kg dose approximates zero-order kinetics for the first hour after administration (possibly rate-limited by absorption) and then describes the same first-order kinetics of the 1 mg/kg dose. These results suggest that the half-time of pulmonary elimination from the FG compartment for rats approximates 45 minutes, versus 6 to 6.5 hours for man. However, as noted for rats, single inhalation or oral exposures in man probably do not result in total body equilibrium, particularly with the fat compartment, and elimination from this compartment may be longer than indicated from these experiments.

Withey and Collins (1980) determined the kinetics of elimination of DCM from the blood of Wistar rats after intravenous administration of 3, 6, 9, 12, or 15 mg/kg DCM given intrajugularly in 1 ml of water. For the four lower doses, the blood decay curves best fitted a first-order two-compartment model, but the highest dose fitted a first-order three-compartment model. Withey and Collins suggested that the apparent change from a two- to three-compartment system occurred as a consequence of a "difference in biological response to

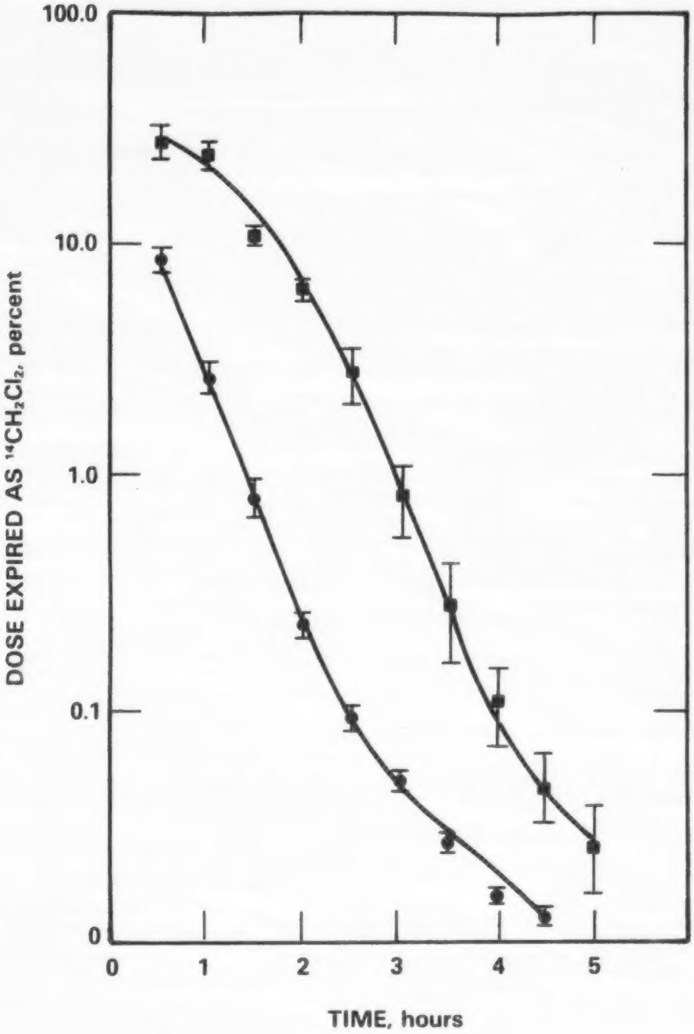

Figure 4-4. Pulmonary elimination of ^{14}C-DCM following oral administration to rats of a single dose of 1 or 50 mg/kg ^{14}C-DCM (squares). Ordinate is percent dose administered during 0.5-hour collection periods with means ± SEM for groups of three rats. SEM is the standard error of the mean.

the dose either as a consequence of the magnitude of the dose or, in some cases, due to the varying response of different animals to the same dose." This interpretation is probably true because the k_e (rate constant for elimination from blood out of body, principally via pulmonary excretion and/or metabolism) was relatively high for the lower doses and consistent with a half-time of body elimination of only 4.5 min. Any significant equilibration of blood DCM with the fat compartment was very unlikely during these experimental circumstances. Further support for limited distribution of DCM is the reported kinetics for the highest dose (15 mg/kg) that fit a three-compartment system. In this case, k_e was 0.09/min, that is, a half-time of body elimination of 7.7 min. This result reflects the fact that more time was required for equilibration into fat to occur. The half-time of elimination from the fat compartment was given as 31.5 min, although the rate constant k_{31} [movement of DCM from the fat compartment to the central compartment (blood)] was given as 0.006/min, indicating a half-time of 115 min. The volume of distribution (Vd) of DCM was calculated as 48.8 ml or about 12 percent of body weight, which was surprisingly low for both a water- and lipid-soluble compound that is known to diffuse into all the major organ systems.

4.2 METABOLISM OF DCM

DCM is known to be metabolized in man to CO and in experimental animals to CO and CO_2. The CO production results in an elevation of blood COHb content, from which CO dissociates in the lung, followed by elimination of CO. Experiments in man and animals have shown that the metabolism of DCM is dose dependent and limited by hepatic enzyme saturation.

4.2.1 Evidence for Metabolism to Carbon Monoxide

Before 1972, most of the absorbed dose of DCM in man was thought to be excreted unaltered in exhaled air, while a small amount was found in the urine (MacEwen et al., 1972; DiVincenzo et al., 1972; Heppel et al., 1944). Metabolism of DCM to CO was not known to occur. However, in 1972, Stewart et al. discovered that the COHb concentration in blood increased in persons exposed to 200 to 1000 ppm DCM (695 to 3474 mg/m^3) for 1 to 2 hours. The COHb levels continued to rise after cessation of exposure and decreased more slowly than when similar levels of COHb were induced by breathing CO. Stewart and his associates (1972a,b) proposed that CO was the end product of DCM metabolism. In the following year, Fodor et al. (1973) showed that COHb blood levels from DCM exposure were further elevated by concomitant exposure to diiodomethane and dibromomethane, thus indicating that these dihalomethanes also are metabolized to CO. At first, this unique halocarbon metabolism was not generally accepted because the increased COHb levels might reflect a change in the rate of endogenous CO production or excretion associated with heme degradation by the microsomal hepatic heme oxygenase system in man (Coburn, 1973; Tenhunen et al., 1969). However, Stewart et al. (1972a,b, 1973) observed no evidence of an enhanced metabolism of hemoglobin in their subjects exposed to DCM, and the subjects did not excrete increased amounts of urobilinogen in their urine either during or after exposure.

A second hypothesis for the origin of the excess COHb postulated a DCM-induced conformational change in hemoglobin, which increased CO affinity. Hence, more COHb would be formed, with a longer biological half-life, from endogenous and exogenous CO sources at the same ambient concentrations. Settle (1971) had shown that xenon and, to a much larger degree, cyclopropane bind to myoglobin and increase CO affinity. Following this observation, Nunes and Schoenborn (1973) used X-ray diffraction to demonstrate that DCM also binds to sperm whale myoglobin and suggested that this binding might increase CO affinity. Settle (1975) then investigated the CO binding to human hemoglobin. He observed that CO binding to human hemoglobin in vitro at 37°C is increased in the presence of DCM (1000 ppm, 3474 mg/m^3). The amount of COHb formed at a given CO concentration is doubled. Also, measurement of the P_{50} values of hemoglobin (partial pressure of O_2 or CO at which 50 percent saturation occurs at 37°C) in the presence and absence of DCM (1000 ppm) indicated a six-fold increase in CO affinity. From these observations, Settle (1975) suggested that the increased COHb seen in vivo may be caused by an increase in CO affinity and not by metabolism. More recently, Collison et al. (1977) determined the Haldane affinity constant for CO for both human and rat blood equilibrated at 37°C with air containing only CO and CO plus DCM (10,000 ppm). No difference in the Haldane constants for human blood (mean value of 227) or rat blood (mean value of 179) was found. Negative results also were obtained when the absolute affinity of CO was measured in a nitrogen-DCM atmosphere. Dill et al. (1978) redetermined the P_{50} value for human and rat blood in the presence and absence of CO (2500 ppm) and DCM (800 ppm). DCM was found to have no effect on the P_{50} values; therefore, Dill concluded that DCM does not significantly increase the affinity of hemoglobin for CO.

The biotransformation of DCM to CO and CO_2 has now been confirmed by many metabolism studies. The hepatic metabolism of DCM has been unequivocally shown to be the origin of the CO responsible for increased COHb blood concentrations. Independently, several groups have shown by administering ^{13}C-DCM or ^{14}C-DCM to rats that labeled CO subsequently appears in COHb with essentially the same specific activity (Carlsson and Hultengren, 1975; Miller et al., 1973; Kubic et al., 1974; Zorn, 1975). Furthermore, Fodor and coworkers (1973, 1976) and Kurppa et al. (1981) have demonstrated that rats exposed to CO and DCM singly and in combination showed an additive increase in blood COHb levels (Table 4-8). In addition, many investigators have shown a dose-response relationship between injected or inhaled DCM and increased blood COHb levels in both experimental animals and men (Figures 4-5 and 4-6) (DiVincenzo et al., 1972; Astrand et al., 1975; Fodor et al., 1973; Ratney et al., 1974; Stewart et al., 1973, 1976a,b; Forster et al., 1974; Roth et al., 1975; Ciuchta et al., 1979; Hake et al., 1974).

The extensive metabolism of halogenated hydrocarbons to CO is apparently unique to the dihalomethanes. This metabolism is not observed to any significant extent with chloroform, carbon tetrachloride, methyl chloride, methyl iodide, trichlorofluoromethane, dichlorodifluoromethane, carbon disulfide, formaldehyde, formic acid, or methanol (Miller et al., 1973; Kubic et al., 1974; Fodor and Roscovanu, 1976; Rodkey and Collison, 1977). Fodor and Roscovanu (1976) state, without giving data, that chloroform, bromoform, and iodoform are metabolized to CO in the rat, thus increasing blood COHb levels; this has not been the observation of other investigators. According to Fodor and Roscovanu (1976), of the dihalomethanes, the bromo-iodo-halides are more extensively metabolized to CO than is DCM (Figure 4-7), thus increasing COHb to a significantly greater extent than DCM metabolism (Miller et al., 1973; Kubic et al., 1974; Fodor and Roscovanu, 1976; Rodkey and Collison, 1977).

4.2.2 Evidence for Dose-Dependent Metabolism of DCM: Michaelis-Menten Kinetics

4.2.2.1 Studies in Humans--McKenna et al. (1980) have reported in abstract form kinetic studies carried out in six healthy male volunteers exposed to 200 and 350 ppm DCM (695 and 1216 mg/m^3) during each of two 6-hour exposure periods. These investigators reported that the metabolism of DCM was dose-dependent,

TABLE 4-8. BLOOD CARBOXYHEMOGLOBIN CONCENTRATIONS OF RATS EXPOSED TO CO AND DCM BY INHALATION

Exposure concentration, ppm DCM	Exposure concentration, ppm CO	COHb saturation %
100	(0.5 - 2)[a]	6.2
1000	(0.5 - 2)[a]	12.5
0	100	10.9
100	100	16.4
1000	100	19.0
None	None	0.7 ± 0.2[b]
-	100	8.8 ± 1.9[b]
1000	-	6.2 ± 0.9[b]
1000	100	14.6 ± 1.3[b]

[a] Ambient air CO concentration.

[b] Mean ± standard deviation, N= 5/group.

Source: Fodor et al., 1973; Kurppa et al., 1981.

based on comparison of the kinetics of the two doses during and following exposure. They compared the DCM blood levels and of the concentrations of DCM in expired air. Blood COHb levels and exhaled CO concentrations were less than those expected for the 350 ppm (1216 mg/m^3) exposure. Calculations made of the rate of DCM metabolism during exposures were consistent with Michaelis-Menten kinetics for DCM metabolism.

More recently, DiVincenzo and Kaplan (1981a) evaluated the conversion of DCM to COHb and CO in sedentary, nonsmoking individuals (11 males and 3 females) exposed to DCM levels of 50, 100, 150, or 200 ppm (174, 347, 521, or 695 mg/m^3), for 7.5 hr, or for 7.5 hr daily to 100, 150, or 200 ppm (347, 521, or 695 mg/m^3) for 5 consecutive days. Between 25 and 34 percent of the absorbed DCM was excreted in expired air as CO during and after exposure. The

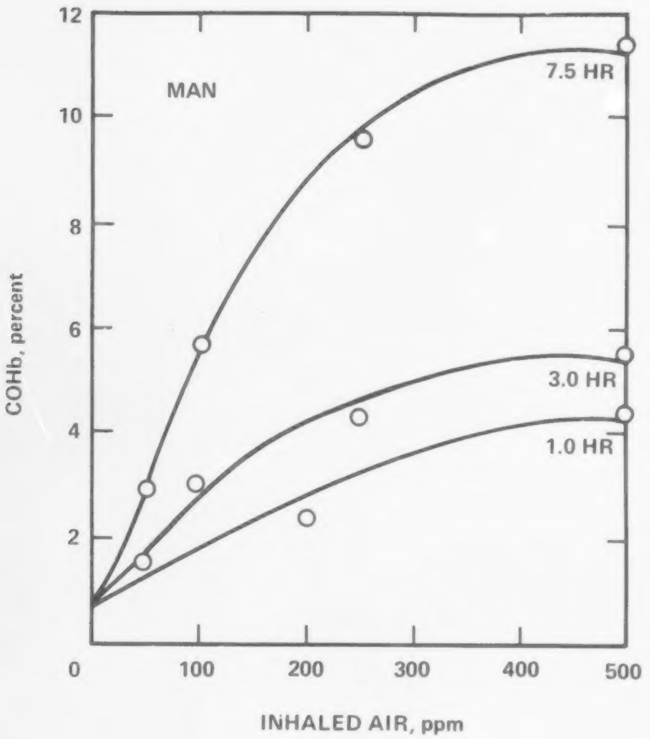

Figure 4-5. Carboxyhemoglobin concentrations in male nonsmokers exposed to increasing concentrations of DCM for 1, 3, or 5 h/day for 5 days. Pre-exposure values averaged 0.8%, but with 3- and 7.5-hour exposures the pre-exposure values were above this baseline value on the mornings following exposure.

Source: Stewart and associates (1972, 1973, 1974).

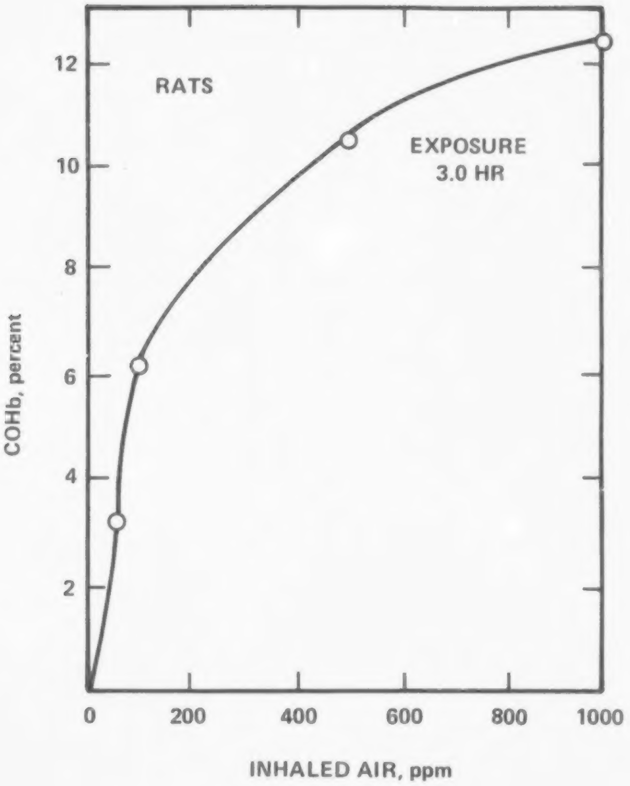

Figure 4-6. Carboxyhemoglobin concentrations in rats after inhalation exposure to increasing concentrations of DCM for single exposures of 3 hours. The values are corrected for pre-exposure COHb concentration and calculated from the data of Fodor et al., 1973.

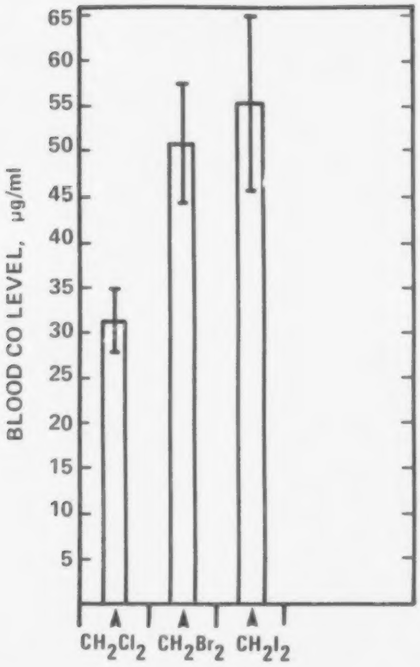

Figure 4-7. Blood CO content of rats after 3-hour inhalation exposure with 1000 ppm dichloromethane, dibromomethane, and diiodomethane, respectively.

Source: Fodor and Roscovanu, 1976.

authors estimated that as much as 70 percent of the inhaled vapor was absorbed; less than 5 percent was expired as unchanged DCM. Materials balance indicates that as much as 70 percent of the amount absorbed may be converted to CO_2. Blood COHb levels increased directly with the magnitude of exposure and did not reach a plateau after 7.5 hr of exposure. At 200 ppm (695 mg/m^3), the peak blood COHb level was 6.8 percent. At the recommended TLV® for DCM (100 ppm; 347 mg/m^3) for 7.5 hr, COHb levels were about 3 percent less than the increase in blood COHb levels produced by an exposure to CO at its recommended TLV® of 35 ppm. DiVincenzo and Kaplan (1981b) also observed that physical exercise resulted in higher blood levels than those found with sedentary individuals. Physical exercise (Table 4-2) increased absorption of DCM, the biotransformation of DCM to CO, blood COHb levels, and pulmonary excretion of CO. Individuals were exposed to 100 ppm DCM (347 mg/m^3) for 7.5 hr. The authors concluded that workers performing physical exercise while exposed to DCM at the recommended TLV® are unlikely to exceed the COHb biological TLV® suggested by the National Institute of Occupational Safety and Health (NIOSH).

4.2.2.2 Studies in Animals--DiVincenzo and Hamilton (1975) provided the first information on the extent to which DCM is metabolized in rats. These investigators injected rats intraperitoneally with ^{14}C-DCM in corn oil and determined fate and disposition of radioactivity in exhaled air, urine, feces, and carcass 1, 8, and 24 hr after single doses ranging from 412 to 930 mg/kg. Volatile compounds in exhaled air were collected, identified, and quantified by GC and radiotracer assay. Recovery of radioactivity was essentially 100 percent 24 hr after administration. About 98 percent of total radioactivity was eliminated in exhaled air, and less than 2 percent was eliminated in urine or feces (Table 4-9). Some 90 percent of injected DCM was eliminated unmetabolized in exhaled air. Most of this elimination (85 percent) occurred within 2 hr. Only 2 percent of the dose was metabolized to CO, 3 percent was metabolized to CO_2, and 1.5 percent was metabolized to an unidentified volatile compound (Table 4-9). These results indicate that less than 7 percent of the dose is metabolized in the rat.

Rodkey and Collison (1977) considered that the small proportion of metabolized DCM found by DiVincenzo and Hamilton (1975) could be caused by the high dose of DCM used, i.e., metabolic transformation of DCM may be limited by the more rapid excretion. Rodkey and Collison carried out balance studies with small doses of ^{14}C-DCM administered to rats (17 mg/kg) by inhalation or

TABLE 4-9. FATE AND DISPOSITION OF ^{14}C-DCM IN RATS (412-430 mg/kg) INJECTED INTRAPERITONEALLY

	Percent of dose (averages)		
	2 hr	8 hr	24 hr
Breath			
Unchanged ^{14}C-DCM	84.5	94.0	91.5
^{14}CO	0.14	1.43	2.15
$^{14}CO_2$	0.55	1.53	3.04
Unidentified ^{14}C	0.40	0.80	1.49
Total	85.59	97.76	98.18
Urine			
Unidentified ^{14}C	<0.01	<0.01	1.06
Feces			
Unidentified ^{14}C	<0.01	<0.01	0.07
Carcass			
Unidentified ^{14}C (mainly in liver)	3.09	2.06	1.53

Source: DiVincenzo and Hamilton, 1975.

intraperitoneal injection. The animals were placed in a closed rebreathing system that trapped CO_2 and CO after conversion to CO_2 by passing through a catalyst bed of Hopcalite®. About 76 percent of ^{14}C-radioactivity was recovered as ^{14}CO (46.9 percent) and $^{14}CO_2$ (28.9 percent). The remaining 24 percent could have been exhaled as unchanged ^{14}C-DCM, because no radioactivity was recovered in carcass tissues. Their results also showed that DCM is directly metabolized to CO without isotopic dilution. The extent of the conversion was surprisingly great at this low dose, and it is independent of the mode of administration. For each mole of DCM metabolized, about 0.5 mole of CO and 0.3 mol of CO_2 were produced.

In a second experiment, Rodkey and Collison (1977) investigated the relationship between dose and extent of metabolism. DCM was administered to rats by intraperitoneal injection or by vaporization of the dose in their closed rebreathing system with a CO trap. DCM doses from 6.8 to 69 mg/kg were given. CO production and DCM disappearance were calculated from the change in gas phase composition, as determined by GC. A control period was used to measure the endogenous rate of CO production. When DCM was added to the system, there was an immediate increase in the initial rate of CO production (about 35 times endogenous for all doses) and a rapid disappearance of DCM from the gas phase (90 percent in 30 minutes). Carbon monoxide continued to be produced for more than 2 hr after nearly complete disappearance of gaseous DCM. Figure 4-8 shows the rates of CO production for various doses of DCM. The initial rates (about 25 μmol hr/kg body weight) are similar for all doses, but for lower doses they progressively decrease after 1 to 2 hr to the endogenous rate as the DCM dose is metabolized. For a very high dose of DCM (69 mg/kg), CO was produced at a nearly constant rate over a 6-hr period (COHb, 44 percent). These observations suggest a saturation of the metabolizing enzymes even at the lowest dose (6.8 mg/kg), giving initially zero-order kinetics followed by first-order kinetics as the DCM concentration in the inhaled air is decreased below enzyme saturation. The total amount of CO produced was related to the dose of DCM. For lower doses, the moles of CO produced per mole of DCM were similar and averaged 0.48; at a high DCM dose (69 mg/kg), the ratio was 0.62 after 10.5 hr of exposure, suggesting that substrate-induced enzyme formation may occur with long exposure to high doses of DCM. Similar results were obtained with germ-free rats, obviating intestinal bacteria as a source of CO. In normal rats, DCM inhibited methane production by intestinal bacteria. The same results were also obtained with dibromomethane, dichloromethane, bromochloromethane, and diiodomethane in respective order of magnitude and rate of CO production.

Rodkey and Collison's (1977) important finding that the metabolism of DCM to CO in rats is rate-limited by enzyme saturation to about 25 μmol/hr/kg body weight explains the seemingly low conversion observed by DiVincenzo and Hamilton (1975) in this species. Recalculation of their data (Table 4-5) gives a DCM metabolism to CO of about 19 μmol/hr/kg body weight. Yesair et al. (1977) obtained comparable results for mice. They administered 1.0 and 100 mg/kg ^{14}C-DCM in corn oil by intraperitoneal injection. Exhaled ^{14}CO, $^{14}CO_2$, and unmetabolized ^{14}C-DCM were trapped (^{14}CO after oxidation with Hopcalite® to $^{14}CO_2$ in aqueous potassium hydroxide and ^{14}C-DCM on coconut

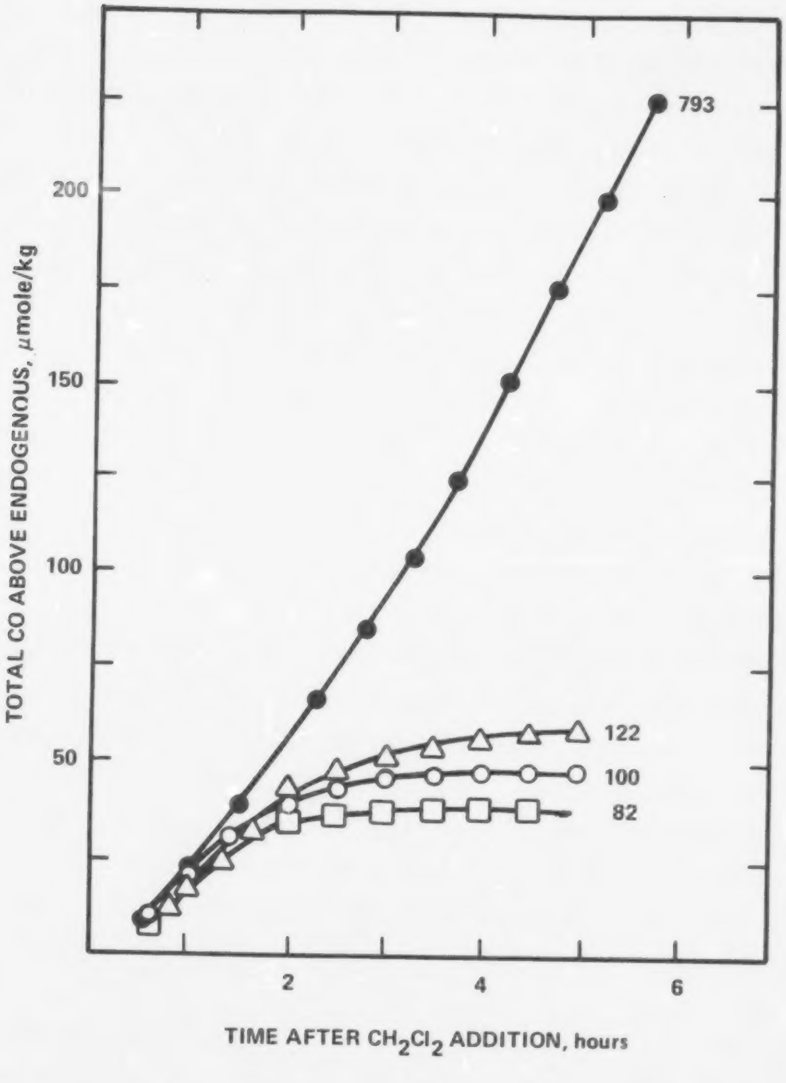

Figure 4-8. Rates of production of CO from DCM given to rats. Each curve represents changes above endogenous CO rate after the dose (in μmoles/kg body weight) was given by inhalation.

Source: Rodkey and Collison, 1977.

charcoal) and quantified by GC and radiotracer assay. The 1 mg/kg dose (11.76 μmole/kg) was quantitatively metabolized to CO (0.45 mol/mol DCM) and CO_2 (>0.50 mol/mol DCM). In the exhaled air collected for 12 hr, the larger dose (11.76 μmol/kg) yielded 4.7 μmol/kg of unmetabolized ^{14}C-DCM (40 percent dose), 0.20 mol ^{14}CO, and 0.25 mol $^{14}CO_2$. Hence, in mice under these experimental conditions, a 12 μmol/kg dose of DCM (1 mg/kg) does not saturate the metabolizing enzymes, whereas 1200 μmol (100 mg) DCM/kg saturates the enzymes and is metabolized at a constant rate of about 20 μmol/hr/kg body weight. The remainder of the dose is excreted unchanged in the exhaled air.

More recently, McKenna et al. (1982) exposed rats to 50, 500, and 1500 ppm ^{14}C-DCM (174, 1737, and 5211 mg/m^3) for 6 hours. They also found that the net uptake and metabolism of DCM to CO and CO_2 did not increase in direct proportion to the incremental increase of DCM exposure concentration (Table 4-10). Furthermore, increasing amounts of unchanged DCM in exhaled air were found with increasing exposure concentration (Table 4-10). At the end of the 6-hour exposure, the body burden of DCM was calculated from total radioactivity recovered during the first 48 hours following exposure; the body burdens had not increased to the incremental increase of DCM exposure concentration (Table 4-11). McKenna et al. estimated the amount of DCM metabolized during each inhalation exposure by subtracting the unchanged DCM recovered in expired air (Table 4-10) from the calculated body burdens. The results are summarized in Table 4-11.

Of particular interest is the finding that the percent of body burden metabolized decreased with the increase of body burden or increase of the level of DCM exposure. McKenna et al. plotted the data in Table 4-11 in accordance with the following linear form of the Michaelis-Menten equation:

$$\frac{dC}{dt} = -K_m \cdot \frac{dC}{dt} \Big/ S + V_{max} \tag{4-2}$$

where dC/dt is the μg/kg DCM metabolized during the fixed time of the experiment and S is the exposure concentration in parts per million. Estimates of V_{max} and K_m, as determined from the y-intercept and slope, were used with a computer program of the Michaelis-Menten equation to derive the values $V_{max} = 65.55 \pm 2.54$ mg/kg DCM metabolized and $K_m = 493 \pm 57$ ppm DCM. Deviation from first-order kinetics occurred at about 250 ppm (868 mg/m^3) or one-half K_m. Therefore,

TABLE 4-10. FATE OF ^{14}C-DCM IN RATS AFTER A SINGLE 6-HOUR INHALATION EXPOSURE

Parameter measured	% body burden ($\bar{x}$ ± S.D., n=3)		
	50 ppm	500 ppm	1500 ppm
Expired CH_2Cl_2	5.42 ± 0.73	30.40 ± 7.10	55.00 ± 1.92
Expired CO_2	26.20 ± 1.21	22.53 ± 4.57	13.61 ± 1.20
Expired CO	26.67 ± 3.00	18.09 ± 0.81	10.23 ± 1.68
Urine	8.90 ± 0.39	8.41 ± 0.90	7.20 ± 0.74
Feces	1.94 ± 0.19	1.85 ± 0.68	2.33 ± 0.05
Carcass	23.26 ± 1.62	11.65 ± 1.87	7.24 ± 0.65
Skin	6.85 ± 1.62	6.72 ± 0.13	3.97 ± 0.15
Cage wash	0.75 ± 0.33	0.24 ± 0.23	0.43 ± 0.15

Source: McKenna et al., 1982.

zero-order kinetics with saturation of DCM metabolizing capacity can be expected at two to three times K_m, or between 1000 and 1500 ppm of inhaled DCM (3,474 and 5,211 mg/m^3). These values correspond to a body burden of about 100 mg/kg for the rat (Table 4-11); therefore, they are in general accord with the observations of Rodkey and Collison (1977) for the rat and Yesair et al. (1977) for the mouse.

Methods of determining the kinetic behavior of DCM metabolism during or after inhalation exposure are subject to unknown error from the indirect methods of estimating both body burden and amount or rates of metabolism. McKenna and Zempel (1981) investigated the kinetics of DCM metabolism in the rat after doses of 1 or 50 mg/kg ^{14}C-DCM. Table 4-12 gives the disposition of DCM after single oral doses in terms of ^{14}C-equivalent.

The results of this study clearly indicate that the metabolism of DCM in rats after oral dosing is dose dependent. Rats given a 1 mg/kg dose metabolized a greater percentage of the oral dose (88 percent) than those given a 50 mg/kg dose (28 percent). Moreover, the rates of pulmonary elimination of unmetabolized DCM (Figure 4-4) and of the metabolites CO_2 and CO were first order and were essentially unaffected by the dose despite large differences in the amounts of DCM and of metabolites excreted. Therefore, the dose-dependent fate of DCM is caused by the saturation of metabolic pathways. Comparison of the near-saturating inhalation body burden (100 mg/kg) (Table 4-11) shows a fair correspondence.

TABLE 4-11. BODY BURDENS AND METABOLIZED ^{14}C-DCM IN RATS AFTER INHALATION EXPOSURE TO ^{14}C-DCM

Exposure concentration	Total body burden,[a] mgEq ^{14}DCM/kg	Metabolized ^{14}DCM[a] mgEq ^{14}DCM/kg	Metabolized ^{14}C-DCM, %
50 ppm	5.53 ± 0.18	5.23 ± 0.32	94.6
500 ppm	48.41 ± 4.33	33.49 ± 0.33	69.2
1500 ppm	109.14 ± 3.15	49.08 ± 1.37	45.0

[a] Values are mean ± standard deviation; number of animals in each group = 3.

Source: McKenna et al., 1982.

TABLE 4-12. FATE OF DCM IN RATS 48 HOURS AFTER SINGLE ORAL DOSES

Parameter Measured	Percent ^{14}C-DCM dose by dose concentration[a]	
	1 mg/kg	50 mg/kg
Expired CH_2Cl_2	12.33 ± 1.43	72.09 ± 0.07
Expired CO_2	35.01 ± 0.85	6.33 ± 0.39
Expired CO	30.92 ± 1.67	11.87 ± 0.07
Urine metabolites	4.52 ± 0.05	1.96 ± 0.05
Feces	0.93 ± 0.02	0.25 ± 0.02
Carcass	5.84 ± 0.24	2.40 ± 0.24
Skin	1.56 ± 0.05	1.15 ± 0.06
Cage wash	0.53 ± 0.04	0.08 ± 0.01

[a] Values are mean ± standard deviation. Number of animals in each group = 3.

Source: McKenna and Zempel, 1981.

In summary, the metabolism of DCM is dose dependent and follows Michaelis-Menten kinetics in the rat and mouse. In these species, saturation of metabolic capacity with zero-order kinetics (about 25 µmol/kg/hr) occurs at about 50 to 100 mg/kg orally or 1000 ppm DCM (3,474 mg/m^3) in inspired air for an inhalation period of 6 hours. At oral doses of 1 mg/kg or at 50 ppm (174 mg/m^3) inhalation, 90 percent is metabolized, and at enzyme saturation doses 30 to 45 percent is metabolized. Dose-dependent metabolism of DCM also occurs in man; at 100 to 200 ppm (347 to 694 mg/m^3) inhalation concentration, 50 to 60 percent of the body burden is metabolized, as judged from retention values of DCM determined in man (Table 4-1). Experimental studies in man suggest that as much as 95 percent of the absorbed dose may be metabolized at these exposure levels (DiVincenzo and Kaplan, 1981a).

4.2.2.3 Effect of Dosing Vehicle--Pritchard and Angelo (1982) have described a physiologically based pharmacokinetic model (Bishoff model) for mice and have used it to simulate the distribution, metabolism, and elimination of DCM after both acute and chronic dosing. Preliminary results indicate that the kinetics depend on the route and vehicle used for administration. Administration of DCM in water by oral gavage or by intravenous injection yields similar blood and tissue profiles; administration in 50 percent polyethylene glycol/water shows a rapid blood elimination and a slow liver elimination, while oral gavage with corn oil as a vehicle shows a slower rate of tissue clearance than that found with a water vehicle.

Withey et al. (1983) have investigated the absorption of DCM in fasting rats following oral gavage of equivalent doses (125 mg/kg) in 4 ml of water or corn oil. The post-absorptive peak blood concentration averaged three times higher for a water vehicle than for corn oil (121 µg/ml versus 44 µg/ml), while the time to peak blood concentration averaged three times longer for corn oil than for the water vehicle (16.3 min versus 5.2 min). Aside from these differences in vehicle-mediated absorption, absorption was apparently rapid with both vehicles and occurred to a comparable extent because the ratio of the areas under the blood concentration curves averaged 1.25 for water:oil. The post-absorption kinetics of blood elimination of DCM associated with the corn oil vehicle also was extended ($t_{\frac{1}{2}}$ was 49 min for oil versus 32 min for water). These vehicle-related differences in absorption, kinetics of elimination, and tissue clearance are probably related to altered transfer of DCM from oil or water across the gastrointestinal (GI) tract mucosa. In contrast to aqueous absorption into the portal system and then to the liver, corn oil and other lipids are extensively transported via the mucosal lymphatic system, which slowly drains by way of the left lymphatic thoracic duct at the junction of the internal jugular and subclavian veins and hence into the systemic circulation via the superior vena cava. The absorption, first-pass metabolism, tissue distribution, and elimination kinetics are probably greatly affected by the volume of lipid vehicle as well as its nature when introduced into the stomach of a rat. While these considerations are unlikely to affect the pharmacokinetics of DCM in man in any practical way, they are of importance in relation to the modes of dosing employed in long-term carcinogenicity tests of DCM and other lipophilic compounds.

4.2.3 Enzyme Pathways of DCM Metabolism

Figures 4-9 and 4-10 summarize current knowledge of the enzyme pathways involved in the biotransformation of DCM and other dihalomethanes. The scheme is based on studies *in vivo* and *in vitro* with hepatocytes and with microsomal and cytosolic preparations. The preponderance of evidence from *in vivo* experiments indicates that these enzyme pathways are unique to the dihalomethanes and give rise to both CO and CO_2 in nearly equimolar amounts. However, CO is an end product of microsomal oxidation, while CO_2 is an end product of cytosolic enzyme systems via the metabolism of formaldehyde and formic acid (Figure 4-9).

4.2.3.1 Microsomal Oxidation--The primary reaction of the dihalomethanes appears to be an oxidative dehalogenation first described by Kubic and Anders (1975). These workers found that DCM and other dihalomethanes were metabolized by rat liver microsomal fractions to CO with inorganic halide release. The system required both nicotinamide adenine dinucleotide phosphate (NADP), reduced (NADPH), and molecular O_2 for maximal activity. These experiments were carried out in a closed vessel, substrates were added without carrier solvent, and CO was determined by the gas chromatographic headspace method. With dibromomethane as the substrate, 3.6 mol of bromide were produced per mole of CO. In the absence of NADPH, microsomal fractions dehalogenated the methanes without CO formation. Anaerobic conditions substantially reduced the rate of conversion, although some CO formation (20 percent maximal) occurred. Equimolar substrate concentrations of dichloromethane, bromochloromethane, dibromomethane, and diiodomethane added to microsomes produced the least

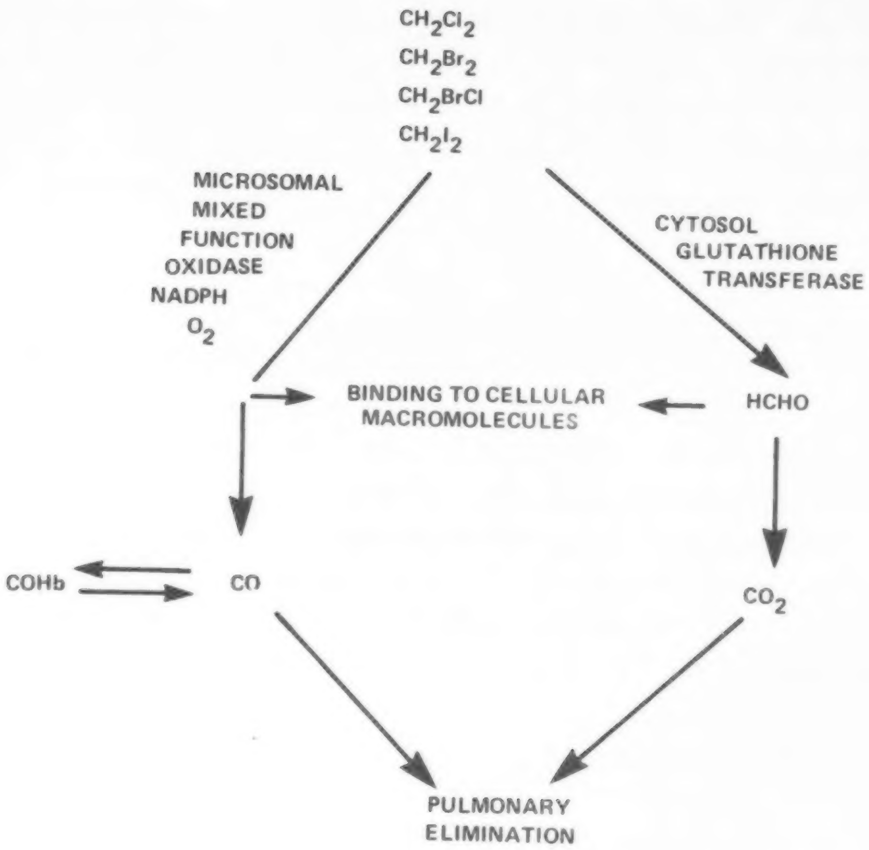
CH_2Cl_2
CH_2Br_2
CH_2BrCl
CH_2I_2
MICROSOMAL MIXED FUNCTION OXIDASE
NADPH
O_2
CYTOSOL GLUTATHIONE TRANSFERASE
BINDING TO CELLULAR MACROMOLECULES
HCHO
COHb
CO
CO_2
PULMONARY ELIMINATION

Figure 4-9. Enzyme pathways of the hepatic biotransformation of dihalomethanes.

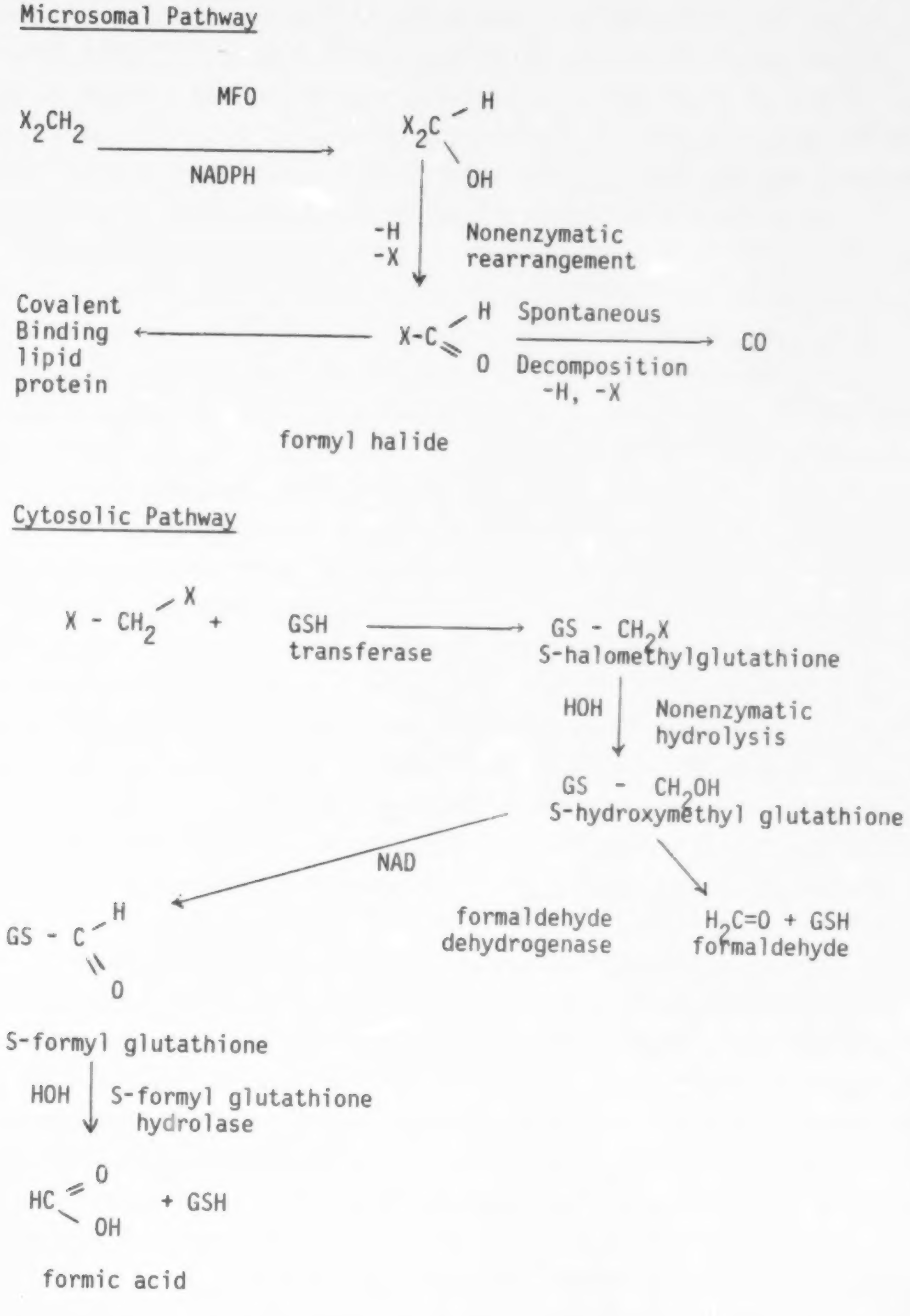
Microsomal Pathway
X2CH2
MFO
NADPH
X2C H OH
-H
-X
Nonenzymatic
rearrangement
Covalent
Binding
lipid
protein
X-C H O
Spontaneous
Decomposition
-H, -X
CO
formyl halide
Cytosolic Pathway
X - CH2 X
+
GSH
transferase
GS - CH2X
S-halomethylglutathione
HOH
Nonenzymatic
hydrolysis
GS - CH2OH
S-hydroxymethyl glutathione
NAD
GS - C H O
formaldehyde
dehydrogenase
H2C=O + GSH
formaldehyde
S-formyl glutathione
HOH
S-formyl glutathione
hydrolase
HC O OH
+ GSH
formic acid

Figure 4-10. Proposed reaction mechanisms for the metabolism of dihalomethanes to CO, formaldehyde, formic acid, and inorganic halide.

Source: Ahmed et al., 1980.

amount of CO for dichloromethane, while diiodomethane yielded the greatest amount (seven times DCM). Liver microsomes were 5 times more active than lung microsomes and 30 times more active than kidney microsomes. Hogan et al. (1976) also found that DCM was converted to CO by rat liver microsomes requiring aerobic conditions and an NADPH generating system. These workers noted a high correlation between _in vitro_ CO production and microsomal cytochrome P_{450} content.

Further evidence of the participation of the P_{450} mixed-function oxidase system in the metabolism of dihalomethanes is the observation that dibromomethane and dichloromethane added to microsomal cytochrome P_{450} preparations produce type 1 binding spectra (Kubic and Anders, 1975; Cox et al., 1976). However, Cox et al. (1976) found that the affinity for P_{450} is less for DCM (K_S, 10 mM) than for chloroform (K_S, 3 mM) or carbon tetrachloride (K_S, 1.5 mM) although carbon tetrachloride and chloroform do not give rise to significant amounts of CO _in vivo_ (Miller et al., 1973; Kubic and Anders, 1975; Rodkey and Collison, 1977). Both Kubic and Anders (1975) and Hogan et al. (1976) found that _in vivo_ phenobarbital pretreatment induced additional _in vitro_ CO production, while cobaltous chloride, which depletes microsomal cytochrome P_{450}, reduced microsomal CO production. Furthermore, SKF 525A, ethylmorphine, and hexobarbital (type 1 substrates) inhibited _in vitro_ microsomal conversion of dibromomethane to CO (Kubic and Anders, 1975).

Kubic and Anders and coworkers (Kubic and Anders, 1978; Ahmed and Anders, 1978; Stevens and Anders, 1978, 1979) have studied the mechanism of the reaction of DCM with deuterium and with $^{18}O_2$. The reaction shows a prominent deuterium isotope effect with a comparative rate of about 12 percent of that of the hydrogen isotope, indicating that carbon-hydrogen bond breakage is the rate-limiting step. Studies with $^{18}O_2$ showed that the oxygen appearing in CO is derived from molecular oxygen rather than from water. On the basis of these studies, Kubic and Anders and coworkers proposed the reaction mechanism shown in Figure 4-10. P_{450}-mediated hydroxylation of dihalomethanes yields the intermediate, hydroxydihalomethane (X_2CHOH), which spontaneously rearranges to a formyl halide (XCHO) with the loss of one halogen atom. The resulting formyl halide is known to readily decompose to yield CO.

4.2.3.2 Cytosolic Pathways--In addition to metabolism to CO, DCM is also converted to formaldehyde, formic acid, inorganic halide, and CO_2. Kuzelova and Vlasak (1966) detected formic acid in the urine of DCM-exposed workers and suggested that DCM was metabolized via formaldehyde to formic acid. Originally, Heppel and Porterfield (1948) reported the conversion of dibromomethane and bromochloromethane to stoichiometric amounts of formaldehyde and inorganic halide by a 9000-g supernatant fraction of rat liver and by liver slices and homogenates. The system did not require O_2, but it was glutathione dependent. Kubic and Anders (1975) more recently confirmed these findings and localized this metabolic pathway to the cytosol. Ahmed and Anders (1976, 1978) extended these findings and showed that the cytosol system is a glutathione transferase that is found only in the liver; it requires no cofactor other than glutathione (cysteine is not a substitute) and is not inducible by phenobarbital or by repeated exposure to DCM or dibromomethane. Furthermore, the reaction was inhibited by reagents that react with sulfhydryl groups, such as diethyl maleate and parachloromercuribenzoate, as well as known substrates for glutathione transferases. The substrate order of activity is diiodo > dibromo = bromochloro > dichloromethane; this order is the same as that found for oxidative dehalogenation by Kubic and Anders (1975). This pathway probably does not contribute to CO production via metabolism of formaldehyde to CO because formaldehyde administration does not produce an increase of COHb in animals or humans (Kubic et al., 1974; Rodkey and Collison, 1977; Kasselbart and Angerer, 1974).

The cytosolic pathway has been investigated in detail by Ahmed and Anders (1976, 1978), who have proposed the reaction sequence shown in Figure 4-9. These workers observed that both dibromomethane and bromochloromethane have identical kinetic constants, suggesting that the initial and rate-limiting step is a displacement of halide, with glutathione interaction, to give the conjugate S-halomethylglutathione. This conjugate is postulated to undergo rapid nonenzymatic hydrolysis to produce S-hydroxymethylglutathione and thus to yield formaldehyde and regenerate glutathione. Because the addition of nicotinamide adenine dinucleotide (NAD^+) to incubation mixtures decreased yields of formaldehyde (Ahmed and Anders, 1976), the postulated intermediate, S-halomethylglutathione, a known substrate of the hepatic cytosolic enzyme formaldehyde dehydrogenase, also undergoes conversion to S-formylglutathione, and with enzymatic hydrolysis (S-formyl glutathione hydrolase) yields formic acid and regenerated glutathione. Ahmed and Anders (1978) found that removal of formaldehyde dehydrogenase from the cytosolic fraction by precipitation with ammonium hydroxide resulted in production of only formaldehyde. Thus, the proposed reaction sequence for the metabolism of dihalomethanes accounts for the experimentally observed stoichiometric ratio of two inorganic halides formed to one formaldehyde and the requirement (but nonconsumption) of glutathione. Furthermore, the sequence serves as a detoxification mechanism for dihalomethanes that depend on cellular availability of glutathione, and the cellular availability of NAD^+ determines the ratio of the products (formaldehyde/formic acid) formed.

4.2.3.3 Carbon Dioxide Formation--The cytosolic glutathione transferase system for dehalogenation of dihalomethanes with the production of formaldehyde and formic acid appears to be the major source of CO_2 as an end product of the metabolism of DCM in the intact rodent (Rodkey and Collison, 1977; DiVincenzo and Hamilton, 1975; Yesair et al., 1977; McKenna et al., 1982). Neely (1964) has demonstrated that formaldehyde is almost quantitatively metabolized to CO_2. When Neely injected ^{14}C-formaldehyde ($^{14}CH_2O$) intraperitoneally into rats at dose levels of 0.25 and 2.5 µmol/kg body weight, he recovered 82 percent of the dose as CO_2 in exhaled air collected over a 24-hour period. Peak concentrations of $^{14}CO_2$ in exhaled air occurred 1 hour after administration. Neely suggested that the formation of CO_2 occurred from formaldehyde entering the 1-C metabolic pool with transfer to glycine by the folic acid cycle to give serine. Transamination of serine to pyruvate provides entry to the tricarboxylic acid cycle and completes the oxidation to CO_2. In support of this metabolic route, small amounts of ^{14}C-labeled serine and methionine were found in the urine. However, when ^{14}C-DCM was administered to rats, no $^{14}CH_2O$ was detected in the breath, serum, or tissues 2 hours later, although substantial changes in tissue formaldehyde content were noted (Rodkey and Collison, 1977; DiVincenzo and Hamilton, 1975).

An additional source of CO_2 production from the metabolism of dihalomethanes is the *in vivo* oxidation of CO to CO_2. Carbon monoxide is metabolized to CO_2 by various animal tissues (Fenn, 1970). Luomanmaki and Coburn (1969) have also demonstrated that $^{14}CO_2$ exists in the expired air of humans breathing ^{14}CO during a 4-hour period in a closed rebreathing system. Carbon monoxide is metabolized by combining with the reduced form of tissue cytochrome oxidase in the presence of a low O_2 tension, and is released as CO_2 (Coburn, 1970). However, the amount of CO_2 generated by oxidation of CO is very small because the rate of conversion is less than 5 percent endogenous CO from heme catabolism (Tzagoloff and Wharton, 1965). On the other hand, the metabolic rate of conversion of CO to CO_2 appears to increase as a function of body stores of CO and thus as a function of blood COHb concentration. In dogs, at 10 to 15 percent COHb, the metabolic conversion rate equals the formation of endogenous CO (Luomanmaki and Coburn, 1969). Increased CO production and elevated COHb that results from catabolism of DCM may stimulate the metabolic production of CO_2 from CO, thus contributing to the total CO_2 produced by DCM metabolism.

4.2.3.4 Pathway Utilization Ratio--The microsomal oxidative dehalogenation and cytosolic glutathione transferase dehalogenation systems (Figure 4-9) account for the CO and CO_2 generated from the metabolism of the dihalomethanes. Because the microsomal system is apparently saturated and rate limiting at low doses (Section 4.2.2), the relative molar amounts of CO and CO_2 produced should provide an index of the activity of the two pathways. However, Yesair et al. (1977) found nearly equimolar amounts of CO and CO_2 with both low and high saturating doses of DCM in mice. For low doses in rats, Rodkey and Collison (1977) found 1.6 times as much CO produced as CO_2, suggesting greater metabolism (at low doses) by the microsomal oxidative pathway. McKenna and Zempel (1981) observed a $CO:CO_2$ ratio of 0.9 for a low oral dose of DCM (1 mg/kg) to rats and a ratio of 1.9 for high saturating dose (50 mg/kg), indicating a greater utilization of the microsomal oxidative pathway at "saturating doses" (Table 4-12). However, McKenna et al. (1982) found no significant preference for either pathway (Table 4-10) for a low inhalation dose of DCM (50 ppm or 174 mg/m^3) and a metabolic saturating inhalation dose (1500 ppm or 5211 mg/m^3). Clearly, the important factors of hepatic content of glutathione and of P_{450} play major roles in the relative utilization of the two pathways of metabolism.

Though it has been observed that equimolar doses of dibromomethane and diiodomethane produce COHb concentrations greater than those produced by DCM in rats (Fodor and Roscovanu, 1976; Roth et al., 1975; Rodkey and Collison, 1977), no information is available on the ratio of CO to CO_2 produced by these compounds in microsomal preparations (Kubic and Anders, 1975) and in cytosol preparations (Ahmed and Anders, 1976). The use of isolated hepatocytes may prove useful and avoid many of the difficulties inherent with whole animal experiments.

4.3 DCM-INDUCED CHANGES IN HEPATIC ENZYMES

The metabolism of the dihalomethanes by the microsomal oxidative dehalogenation pathway (but apparently not the cytosolic pathway) can be modulated by inducers of the microsomal mixed-function oxidase system. Pretreatment of animals with phenobarbital was found by some workers to increase blood COHb levels and microsomal production of CO (Kubic et al., 1974; Kubic and Anders, 1975; Hogan et al., 1976; Stevens et al., 1980), but other investigators found no effect or observed a decrease (Miller et al., 1973; Roth et al., 1975). Roth et al. (1975) suggested that enhanced metabolism may be initially induced by phenobarbital, but the resulting increase in local microsomal levels of CO may be sufficient to inhibit cytochromic P_{450} oxidative dechlorination. Heppel and Porterfield (1948) also reported that repeated administration of bromochloromethane to rats led to an increased rate of dehalogenation. However, Haun et al. (1972) found that continuous exposure of mice to 100 ppm DCM (347 mg/m^3) for 4 to 12 weeks decreased the hepatic cytochrome P_{450} content.

Daily exposures for shorter periods do not appear to influence hepatic P_{450} content, but they do modify the activity of other enzyme systems. Kurppa and Vainio (1981) exposed rats at 500 and 1000 ppm DCM (1737 and 3474 mg/m^3) 6 hr/day for 5 and 10 days. They reported no change in hepatic P_{450} content but they observed a marked decrease of NADPH-cytochrome c (35 percent) and a twofold increase in UDP-glucuronosyl transferase. The hepatic glutathione content remained unchanged. Toftgard et al. (1982) exposed rats to 500, 1500, and 3000 ppm DCM (1737, 5211, and 10,422 mg/m^3) 6 hr/day for 3 days and found no change in hepatic P_{450} content, but a dose-related increase of microsomal metabolism of biphenyl and of benzopyrene was noted. These changes were postulated to be caused by a change in the proportions of different cytochrome P_{450} isozymes resulting from DCM-inducing effect on some specific forms of cytochrome P_{450}. In contrast to these observations, Pritchard et al. (1982) recently administered DCM to male mice for 3 days by gavage (5, 50, 100, 250, 500, 1000 mg/kg doses in corn oil) and found no significant changes in hepatic weight, microsomal protein, P_{450}, cytochrome b_5 contents, or activities of aminopyrene N-demethylase and biphenyl 4-hydroxylase. Furthermore, 28 days of administration of DCM in drinking water, providing daily doses ranging from 5 to 1000 mg/kg, also did not affect these parameters or hepatic glutathione content.

4.4 COVALENT BINDING TO CELLULAR MACROMOLECULES

The likelihood of significant covalent binding of reactive metabolites from the metabolism of DCM to cellular macromolecules depends on the postulated reactive intermediates: formyl chloride from microsomal oxidative metabolism and S-chloromethyl glutathione and formaldehyde from cytosolic metabolism (Figure 4-10). These compounds may be capable of acylating cellular nucleophiles. S-chloromethyl glutathione is structurally similar to the reactive bis-halomethyl ethers.

Anders et al. (1977) have studied the extent and pattern of binding to microsomal lipid and protein after aerobic incubation of rat hepatic microsomes with ^{14}C-DCM. Table 4-13 shows that metabolites of DCM become covalently bound to both microsomal protein and lipid under conditions optimal for metabolism of DCM to CO. Furthermore, microsomes from rats pretreated with phenobarbital showed increased binding. Thus, the formyl chloride intermediate may either acylate macromolecules or decompose to CO.

Cunningham et al. (1981) have investigated covalent binding of intermediates from the metabolism of ^{14}C-DCM by rat hepatocytes. Rat hepatocytes in suspension have been shown to metabolize DCM and other dihalomethanes to CO (Stevens et al., 1980). The results of irreversible binding of ^{14}C-DCM to cellular macromolecules in this system, in comparison to ^{14}C-carbon tetrachloride, are given in Table 4-14.

The covalent binding of carbon tetrachloride to lipids and protein was greatly enhanced in the absence of O_2 and was almost eliminated in the presence of O_2 (Table 4-14). In contrast, binding of DCM and trichloroethylene was enhanced in the presence of oxygen; this is consistent with the microsomal oxidative metabolism of these compounds to an epoxide and formyl chloride, respectively. Glutathione-depleted hepatocytes showed markedly decreased covalent binding of ^{14}C-DCM to both lipids and protein, suggesting inhibition of cytosolic DCM-glutathione conjugation with a decreased production of reactive S-chloromethyl glutathione and of formaldehyde. However, at least part of the decrease in binding may be caused by diethylmaleate inhibition of microsomal oxidation (Stevens et al., 1980) as well as by diethyl maleate depletion of cellular glutathione. Phenobarbital pretreatment also markedly decreased binding from ^{14}C-DCM hepatocyte metabolism. The explanation of this effect is not readily apparent because phenobarbital is not known to deplete cellular glutathione, but it does increase DCM microsomal oxidative metabolism to CO in hepatocytes

TABLE 4-13. *IN VITRO* COVALENT BINDING OF 14C-DCM TO MICROSOMAL PROTEIN AND LIPID

Conditions	nmol ^{14}C bound/mg/min ± SD Protein	 Lipid
Normal rat microsomes	0.24 ± 0.02	0.27 ± 0.02
Microsomes from phenobarbital-treated rats	0.57 ± 0.03	1.97 ± 0.19

S.D. = standard deviation.

Source: Anders et al., 1977.

TABLE 4-14. COMPARATIVE COVALENT BINDING OF DCM, CARBON TETRACHLORIDE (CCl_4), AND TRICHLOROETHYLENE (TCE) TO LIPID AND PROTEIN IN RAT HEPATOCYTES

	Atmosphere[a] (O_2/N_2)		Glutathione Depletion[b], %		Phenobarbital[c] induction, %	
Substrate	Protein	Lipid	Protein	Lipid	Protein	Lipid
$CC\ell_4$	0.09	0.06	100	100	145	120
TCE	9.63	5.08	74	74	288	238
DCM	8.53	11.96	44	38	44	40

[a] Ratio of covalent binding observed under oxygen and nitrogen atmosphere. Values are the mean of three to six determinations for each condition.

[b] Thirty minutes prior to hepatocyte isolation, rats were treated intraperitoneally with 0.6 ml/kg of diethylmaleate. Values are expressed as the mean percent of binding observed in hepatocytes from untreated rats, with N=5.

[c] Rats were pretreated with three consecutive daily doses of phenobarbital (80 mg/kg, intraperitoneally) beginning 4 days before isolation of hepatocytes. Values are expressed as the mean percent of binding observed in hepatocytes from untreated rats, with N=5.

Source: Cunningham et al., 1981.

(Stevens et al., 1980). Perhaps the most important observation made by Cunningham et al. (1981) was that ^{14}C-DCM metabolism by isolated hepatocytes did not result in the alkylation of the nucleic acids RNA or DNA, whereas metabolites of carbon tetrachloride and trichloroethylene labeled nucleic acids under the conditions of the experiment.

Reynolds and Yee (1967) studied labeling patterns of ^{14}C-DCM and ^{14}C-formaldehyde in rat liver after in vivo injection. They found similar patterns of labeling of DCM and its metabolite, formaldehyde. Binding occurred most often at the amino acid locus corresponding to serine and on the acid-soluble cell constituents; smaller amounts occurred in lipid and nucleic acids. However, formaldehyde or formic acid can directly combine with tetrahydrofolic acid and consequently be incorporated into de novo nucleic acid synthesis; therefore, the association of ^{14}C-activity with nucleic acids does not necessarily indicate covalent binding.

4.5 KINETICS OF CARBOXYHEMOGLOBIN FORMATION

Blood COHb accumulates when the amount of endogenous or exogenously derived CO in the body exceeds that of pulmonary elimination. Pulmonary elimination of CO is a first-order process that involves the exchange of hemoglobin-bound CO with oxygen and the diffusion of CO across capillary and alveolar endothelium into alveolar lung spaces. In humans, the half-time of elimination of CO is 4 to 5 hours (NIOSH, 1972; Lambertsen, 1974), and in the rat the time is 1.8 to 2.5 hours (McKenna et al., 1982; McKenna and Zempel, 1981). The half-time of pulmonary elimination is independent of the blood COHb concentration but is dependent on factors such as lung function and dysfunction, pulmonary rate and amplitude, regional blood flow, and cardiac function.

Ordinarily, the sole endogenous source of CO, and hence COHb, is the physiologic catabolism of heme by the hepatic microsomal heme oxygenase pathway (Coburn, 1973; Tenhunen et al., 1969). The endogenous rate of CO production from this source in the normal human is about 20 μmol/hr, producing a blood COHb concentration of approximately 0.4 percent. A close linear correlation exists between the molar rate of CO production and the percent COHb saturation; only 10 to 15 percent of the total body CO is not associated with hemoglobin. Most of this 10 to 15 percent is bound to hemoproteins such as myoglobin and

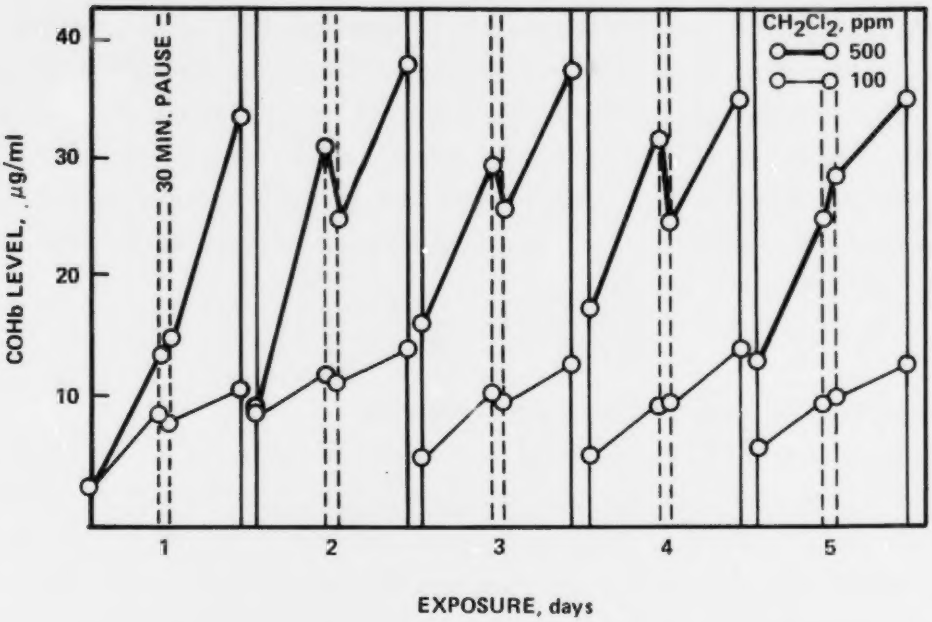

Figure 4-11. Blood COHb level in men during an 8-hour exposure for 5 consecutive days to 500 ppm and 100 ppm DCM. COHb percent saturation is equal to μg CO per ml blood divided by 2.5.

Source: Fodor and Roscovanu, 1976.

heme cytochromes; about 1 percent is dissolved in body water. The rate of endogenous CO production from heme catabolism is markedly inhibited by exogenous CO sources producing COHb levels of approximately 12 percent saturation, suggesting that blood levels of 12 percent saturation are sufficient to inhibit, by CO binding, hepatic microsomal oxidase systems involved in hemoglobin degradation to CO (Coburn, 1970). The half-life of COHb (4 to 5 hours) arising from heme catabolism or exogenous environmental CO (e.g., ambient-air CO, tobacco smoking) is decreased by increased alveolar ventilation or increased inspired partial pressure of oxygen (Lambertsen, 1974).

Since Stewart and his associates (1972a,b) reported the remarkable increase of blood COHb (up to 15 percent from 0.6 percent pre-exposure) in persons acutely exposed by inhalation to DCM vapor, numerous investigations of the phenomenon have been made in experimental animals and humans. Studies have sought to determine the dose-response relationship of blood COHb level with DCM air concentration, with duration of exposure, with the time course of COHb blood concentration rise and decline, and with the magnitude of exposure in the industrial setting. Because CO generated from DCM is additive to exogenous environmental CO, DCM exposures at high levels could pose an additional health burden. Of particular concern are smokers who maintain significant constant levels of COHb, i.e., 4.6 to 5.2 percent (Stewart et al., 1974a,b; Kahn et al., 1974) and others who may have increased sensitivity to CO toxicity, such as pregnant women and persons with cardiovascular disease or pulmonary dysfunction. Indeed, Stewart et al. (1972a,b) have noted that exposure to concentrations of DCM that do not exceed the industrial TLV® (100 ppm, 347 mg/m^3) may yield COHb levels exceeding those allowable from exposure to CO (35 ppm, 40.1 mg/m^3; about 5 percent COHb blood concentration).

As previously discussed, the recent findings of DiVincenzo and Kaplan (1981a,b) with sedentary individuals and those engaged in physical exercise while exposed to DCM indicate that blood levels of COHb are increased but are within the biological TLV® recommended by the National Institute for Occupational Safety and Health (1976). The National Institute for Occupational Safety and Health has recommended that the time-weighted average (TWA) exposure to DCM should not produce COHb levels that exceed 5 percent. DiVincenzo and Kaplan (1981a) found that an 8-hour exposure to 150 ppm DCM (521 mg/m^3) in sedentary individuals is equivalent to an 8-hour exposure to 35 ppm CO; either exposure has been shown to result in blood levels of COHb of about 5 percent.

In their companion study in which individuals either exercised or smoked cigarettes during exposure, DiVincenzo and Kaplan (1981b) observed an additive increase in blood COHb concentrations. Individuals were exposed for 7.5 hours to 100 ppm DCM (347 mg/m^3). The effects of exercise on physiological parameters are presented in Table 4-2. Moderate to heavy workloads produced about a twofold increase in blood COHb levels compared to the increase for sedentary individuals.

From a review of the literature, an important observation of DCM-induced COHb is that the blood concentration, although it is DCM-dose dependent and exposure-time dependent, never exceeds 10 to 12 percent in either humans or animals in usual circumstances of free breathing and normal pulmonary function (Figures 4-5, 4-6, 4-11). This limiting blood concentration is clearly determined by the resultant of first-order pulmonary elimination of CO and zero-order kinetics of hepatic DCM metabolism to CO (Section 4.2.2). Because COHb is essentially confined to the blood compartment, a one-compartment open kinetic model with zero-order input can be used to describe the time course of blood COHb as follows:

$$k_o \longrightarrow \boxed{\begin{array}{cc} V & \\ & C \end{array}} \longrightarrow k_e \qquad (4\text{-}3)$$

where V is the volume of the hemoglobin compartment (90 percent CO is distributed in this compartment) (Luomanmaki and Coburn, 1969), k_o is the zero-order rate of COHb formation, k_e is the first-order rate constant for pulmonary elimination of CO from COHb, and C is the the concentration at any time t of COHb formed. Then,

$$C_{COHb_t} = \frac{k_o}{Vk_e} \left[1-e^{-k_e t} \right] \qquad (4\text{-}4)$$

This equation (Wagner, 1975) describes the time course of rising COHb concentration with zero-order formation of COHb. With long periods of exposure to DCM (6 to 8 hours), the relation becomes

$$C_{COHb_t} = k_o/Vk_e \qquad (4\text{-}5)$$

That is, a steady-state plateau concentration of blood COHb is reached (see Figure 4-12). Therefore, maximal blood COHb is mandated when K_o, V, and k_e are constants.

4.5.1 Studies in Humans

In a series of studies, Stewart and his associates (Stewart et al., 1972a,b, 1973, 1974a,b; Forster et al., 1974; Hake et al., 1974) have shown that blood COHb levels achieved in response to DCM exposure are related to the inhaled concentration and to the duration of exposure. Male nonsmokers were exposed to DCM for 1, 2, and 7.5 hours daily, for 5 days per week. Blood COHb levels were determined for daily pre-exposure and post-exposure times. Their data, which are replotted in part in Figure 4-5, indicate that maximum COHb concentrations occur with 400 to 500 ppm DCM (1390 to 1737 mg/m^3) exposure and increase with duration of exposure. Similar results were reported in women nonsmokers exposed 1, 3, and 7.5 hours to 250 ppm DCM (868 mg/m^3) for 5 consecutive days (Hake et al., 1974) and in male volunteers exposed to 100 and 500 ppm DCM (347 and 1737 mg/m^3) daily for 5 days (Fodor and Roscovanu, 1976) (Figure 4-11). In each of these studies, the time course of decay of COHb levels to pre-exposure levels occurred within 24 hours so that a consistent increment in COHb with daily exposure was not observed (Figure 4-11); however, the half-time of COHb disappearance was significantly longer than expected. The longer apparent half-time of pulmonary elimination of CO produced by hepatic metabolism of DCM results from storage of DCM in adipose tissue with conversion to CO continuing subsequent to termination of exposure. In these circumstances, the biological half-life of COHb derived from DCM (10 to 15 hours) is proportional to the body burden of DCM, in contrast to the constant half-life of 4 to 5 hours from CO inhalation (Stewart et al., 1972a,b, 1976a,b; Fodor et al., 1973; Peterson, 1978; Peterson and Stewart, 1970). However, when workplace exposures were simulated, the biologic half-life for COHb was similar to that for CO (DiVincenzo and Kaplan, 1981a).

DiVincenzo and Kaplan (1981a) found that blood COHb saturation in sedentary male volunteers is not attained during a 7.5-hour exposure to DCM at concentrations ranging from 50 ppm (174 mg/m^3) to 200 ppm (695 mg/m^3). Peak blood COHb during the 200-ppm exposure was 6.8 percent. At the TLV® for DCM, the authors estimated that an 8-hours exposure would produce a blood COHb level of about 3 percent. A linear relationship was found between blood COHb

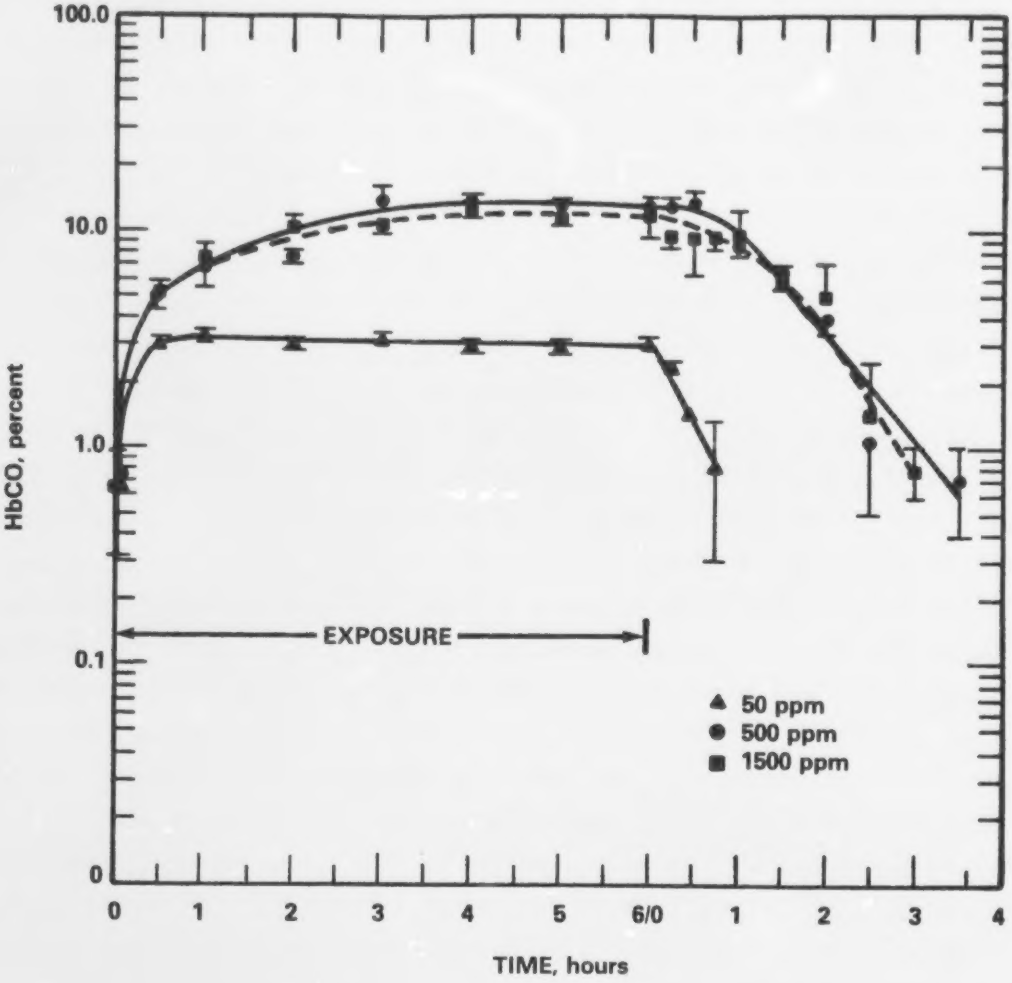

Figure 4-12. Blood HbCO concentrations in rats during and after a 6-hour inhalation exposure to DCM. Each data point is the mean ± standard error for two to four rats.

Source: McKenna et al. (1982).

and DCM exposure concentrations. Repeated exposure to DCM levels of 100, 150, or 200 ppm (347, 521, or 695 mg/m^3) for 7.5 hours daily for 5 consecutive days produced slightly higher blood COHb levels than those found for single exposures. Following the weekend, blood COHb levels returned to pre-exposure levels. Blood COHb declined at a slower rate than DCM in expired breath during post exposure. About 24 hours were required for blood COHb to return to preexposure values.

The findings in a companion study by DiVincenzo and Kaplan (1981b) with smokers and individuals engaged in physical activity indicated that exposure to 100 ppm DCM (347 mg/m^3) may result in slightly higher blood COHb than in sedentary nonsmokers. The investigators concluded that workers performing physical exercise while exposed to DCM at 100 ppm (347 mg/m^3) are unlikely to exceed the COHb biological TLV® recommended by the National Institute for Occupational Safety and Health (1976).

Ratney et al. (1974) studied the blood COHb levels (calculated from alveolar concentrations) of a group of young male adults in their workplace where large quantities of DCM were used (a plastic-film plant). Workroom air concentrations of DCM averaged 200 ppm (695 mg/m^3) with no measurable CO. At the beginning of the work day, blood COHb levels averaged 4.5 percent. After an 8-hour exposure, COHb levels rose to about 9 percent and then declined exponentially to 4.5 percent by the next working day (16 hours later) with an apparent half-time of CO pulmonary elimination of 13 hours. These prolonged half-times for CO in alveolar air and for COHb in blood are difficult to interpret in light of the contrasting results of Stewart et al. (1972a,b) and DiVincenzo and Kaplan (1981a,b). Ratney et al. (1974) reported that pre-exposure levels of CO in expired air and COHb in blood were elevated above the values expected for nonsmokers. Three of the seven individuals that were monitored were not previously exposed to DCM; therefore, it is surprising that the CO levels in their alveolar air and blood COHb values were as high as reported in the paper. Ratney et al. (1974) reported a mean pre-exposure CO concentration in expired air of about 29 ppm. Normal levels of CO in alveolar air are expected to range between 1 and 5 ppm.

The studies of Stewart and associates, Fodor and Roscovanu, and Ratney et al. are consistent in their findings and lead to the following conclusions.

1. COHb derived from CO of DCM metabolism is additive to COHb derived from CO of exogenous sources.

2. Blood COHb concentration attained from DCM metabolism is a function of both DCM inhalation concentration and of exposure duration, with a maximal attainable concentration of 10 to 12 percent COHb.

3. A steady-state blood COHb concentration in sedentary individuals is not attained within 8 hours for single exposures of DCM.

4. The half-time of first-order pulmonary elimination of COHb is 4 to 5 hours, although a longer pseudo half-time of 10 to 15 hours is observed because of continued post-exposure metabolism of DCM, from solvent stored in the fat compartment during exposure.

5. Blood COHb concentrations from multiple daily exposures reach a steady-state level within 3 to 5 days, and the level is only slightly higher than that after a single 8-hour exposure.

These observations on the kinetics of COHb formation during DCM inhalation exposure are consistent with Michaelis-Menten kinetics for DCM metabolism to CO (Section 4.2.2). The data plot of Stewart and associates of Figure 4-5 (7.5-hour exposures) illustrates dose-dependent formation of COHb and is of the form of the Michaelis-Menten equation:

$$\frac{dC}{dt} = \frac{V_{max}\, S}{K_m + S} \qquad (4\text{-}6)$$

where dC/dt is the rate of COHb formation and S is the exposure concentration in parts per million. Estimates of V_{max} and K_m were obtained from plotting the data in accordance with the linear form of the equation.

$$\frac{1/dC}{dt} = \frac{K_m}{V_{max} + S} + \frac{1}{V_{max}} \qquad (4\text{-}7)$$

This form provides values of $V_{max} \cong 15\%$ COHb/7.5 hr and $K_m \cong 200$ ppm (695 mg/m^3) DCM. These values indicate a maximum obtainable COHb blood level in humans caused by DCM exposure in air of about 25 percent, and a saturation of hepatic metabolism of DCM to CO in humans at 400 ppm DCM (1390 mg/m^3) in inhaled air (2 x K_m).

The COHb blood levels resulting from DCM inhalation are dependent on pulmonary function status as well as on metabolism of DCM to CO. Physical activity or exercise during exposure to DCM markedly increases pulmonary absorption and body retention (Section 4.1.3) but tends to diminish the maximum blood COHb level achieved by the end of the exposure period, although higher blood COHb levels are attained 3 to 4 hours after exposure when compared with control sedentary subjects (Stewart and Hake, 1976; Astrand et al., 1975; DiVincenzo and Kaplan, 1981b). These findings may be explained by the decrease in half-life of pulmonary CO elimination affected by the increased pulmonary ventilation rate and increased cardiac output associated with physical activity (Lambertsen, 1974). Conversely, compromised pulmonary function (e.g., adult respiratory distress syndrome, emphysema, and asthma) can be expected to increase COHb blood levels by decreasing pulmonary elimination of CO. A comprehensive kinetic model describing the parameters of absorption, distribution, and elimination of DCM in humans, as well as metabolism to CO and COHb, would be valuable in evaluating the effects of physical activity, pulmonary and cardiovascular diseases, and concomitant environmental xenobiotic exposures, including drugs such as alcohol and barbiturates, of which very little is known. Hake (1979) has developed a computer simulation model, based on the Coburn-Forster-Kane equation, describing the kinetics of COHb formation using the experimental data of Stewart and his associates. He was able to show that physical activity during exposure to DCM led to a lower blood COHb than the sedentary level because while increased ventilation increased DCM uptake, it also increased the pulmonary elimination rate constant (k_e) for CO.

4.5.2 Studies in Animals

The kinetics of COHb formation from DCM exposure elucidated in humans have largely been confirmed in animals. Figure 4-6 shows the relationship between exposure concentration and COHb levels in rats reported by Fodor et al. (1973). For a 3-hour exposure, maximal levels of COHb (12.5 percent) are achieved with about 1000 ppm DCM (3,474 mg/m^3). Fodor and Roscovanu (1976) noted that a 3-hour exposure with 200 ppm DCM (695 mg/m^3) in humans produced COHb levels of about 4.3 percent but they found nearly twice this level in the rat (Figure 4-6); this difference suggests that metabolic capacity for CO formation is greater in rats than in humans. Hogan et al. (1976) found that rats exposed to 440 ppm DCM (1529 mg/m^3) for 3 hours had maximal COHb levels of about 7 percent, and exposure to 2300 ppm (7,990 mg/m^3) produced no further increase. Pretreatment of the animals with phenobarbital increased the rate of rise of COHb levels and the time the maximum level was maintained, but it did not increase the highest level. These investigators suggest that endogenous CO inhibition of the P_{450} metabolizing system by CO binding to the cytochrome may occur at very high levels of DCM exposure. However, their observations are also consistent with the Michaelis-Menten kinetics of hepatic CO formation from DCM, with saturation of metabolic conversion from exposure to 400 ppm (1,390 mg/m^3). Kurppa and Vainio (1981) exposed rats to 500 and 1000 ppm DCM (1737 and 3474 mg/m^3), 6 hr/day for 5 days/week for 1 and 2 weeks. The test results are given in Table 4-15. Since maximal blood COHb levels of 8 to 9 percent occurred with 500 ppm (1737 mg/m^3) exposure and no further increase was obtained with 1000 ppm (3474 mg/m^3) or with longer duration of exposure, these results also indicate that saturation of metabolism of DCM to CO in rats occurs at a DCM exposure concentration of 500 ppm (1737 mg/m^3) or less.

McKenna et al. (1982) exposed rats to 50, 500, and 1500 ppm DCM (174, 1737, and 5211 mg/m^3) for 6 hours; during testing, steady-state conditions were reached between production of CO, maintenance of a given circulation blood COHb concentration, and pulmonary CO excretion. These results are shown in Figure 4-12. The blood percent COHb at steady-state was not linearly related to exposure concentration but reached a maximum of about 12.5 percent. Kinetic parameters for the production of CO and formation of COHb can be estimated on the clear assumption of dose-dependent Michaelis-Menten kinetics (Section 4.2.2). Estimates calculated from the data in Figure 4-12 are $V_{max} \cong$ 13.5 percent COHb/6-hr exposure and $K_m \cong$ 170 ppm DCM (591 mg/m^3). These values indicate a saturating DCM exposure concentration of about 350 ppm (1,216 mg/m^3) ($2 \times K_m$) for the rat.

The determination of Michaelis-Menten kinetics of DCM metabolism to CO and an upper limit to the attainable blood COHb concentration provides an explanation of the observations of Roth et al. (1975) and of Haun et al. (1971, 1972). Roth et al. found that concentrations of COHb in the blood of rabbits after very short exposures (20 minutes) to DCM inhalation concentrations ranging from 2,000 to 12,000 ppm (6948 to 41,688 mg/m^3) were a linear function of DCM exposure concentration. COHb levels were approximately 5.5 percent at 2,000 ppm (6,948 mg/m^3) and 13 percent at 12,000 ppm (41,688 mg/m^3). However, with 4 hours of exposure at about 7,000 ppm (24,318 mg/m^3), steady-state blood COHb concentrations of 14 percent were attained. Phenobarbital pretreatment of the rabbits decreased the blood COHb level achieved with a given DCM exposure, although in parallel experiments, phenobarbital stimulated rabbit microsomal benzene hydroxylase and benzphetamine N-demethylase activity. Therefore, Roth

TABLE 4-15. BLOOD COHb AND HEMOGLOBIN CONCENTRATIONS IN RATS EXPOSED TO DCM[a]

Exposure (ppm)	Carboxyhemoglobin, %		Hemoglobin, g/l	
	Five days	Ten days	Five days	Ten days
None	0.4 ± 0.1	0.4 ± 0.2	155.2 ± 5.5	156.6 ± 6.2
500	8.1 ± 0.6	8.1 ± 0.7	144.0 ± 21.2	155.4 ± 3.5
1000	9.2 ± 0.6	8.5 ± 0.6	151.5 ± 6.0	158.0 ± 4.5

[a]The results are given as mean ± standard deviation of five rats per group.

Source: Kurppa and Vainio, 1981.

et al. (1975) suggested that increased CO production from DCM at the microsome in response to a phenobarbital-induced increase of the cytochrome P_{450} system inhibits further DCM metabolism. A more likely explanation of these results is that the short exposures did not provide sufficient time for the establishment of body equilibrium to inhaled DCM; therefore, even at high inhalation concentrations, the DCM presented to the hepatic-metabolizing system was on the linear portion of the Michaelis-Menten curve.

In a comparison of dogs and monkeys continuously exposed to 25 and 100 ppm DCM (87 and 347 mg/m^3) for 6 to 13 weeks, Haun et al. (1971) found that steady-state levels of blood COHb were maintained throughout the exposure period, and these concentrations were directly proportional to the exposure concentration. However, these concentrations result from low-level exposures; therefore, they are expected to be on the linear portion of the Michaelis-Menten function. Haun et al. noted that dogs had higher steady-state blood concentrations of DCM than monkeys at these inhalation concentrations, but monkeys had the higher COHb blood levels, suggesting that monkeys have a greater hepatic capacity for CO formation.

4.5.3 Comparison of Kinetics of Humans and Rats

The kinetics of DCM metabolism to CO and of COHb formation are strikingly similar for humans and rats. Both species demonstrate Michaelis-Menten dose-dependent kinetics with similar estimates for V_m (15 and 13.5 percent COHb per time unit, respectively) and K_m (200 and 170 ppm DCM, respectively). Hence, the saturating DCM inhalation concentration for the metabolism of DCM to CO and formation of COHb are comparable for the two species (400 versus 350 ppm; 1390 versus 1216 mg/m^3).

The metabolism of DCM involves two pathways: the microsomal oxidative pathway leads to CO, and the cytosolic pathway using glutathione converts DCM to formaldehyde, formic acid, and CO_2. Comparison of the kinetic parameters estimated above for the CO pathway in the rat with those obtained by McKenna et al. (1982) for the total overall (both pathways) metabolism of DCM in these rats (Section 4.2.2) reveals that the $K_m \cong 400$ ppm (1390 mg/m^3) for overall metabolism is about twice that for the CO pathway alone. Furthermore, this figure is an average overall value, suggesting that the cytosolic glutathione-dependent pathway has a much higher K_m value than that of the CO pathway. Andersen (1981) has called the CO metabolism a high-affinity, low-capacity pathway and the glutathione-dependent pathway low-affinity but high-capacity.

4.6 MEASURES OF EXPOSURE AND BODY BURDEN IN HUMANS

In the controlled laboratory setting, estimating the DCM absorbed into the body by comparing inspired and alveolar air concentrations, or by measuring blood levels of DCM and then extrapolating these parameters to body dose, is an imprecise task. However, the goal of this research is to develop a sufficient data base and knowledge of the kinetics of absorption, disposition, elimination, and metabolism of DCM to enable assessment of the body burden of DCM from acute or chronic exposure in the industrial or ambient setting where air concentrations and exposure period vary widely. Air monitoring, an important control measure, cannot be used as a reliable index of body burden. At present, monitoring levels in blood or alveolar air and measuring blood COHb levels are the available approaches to estimating human body burdens from recent exposures. However, in addition to the lack of complete pharmacokinetic knowledge necessary to interpret these determinations as accurate and reliable measures of body burden, the results are also subject to unknown inter-individual variation from factors such as anthropometric differences, metabolism and work load, age and sex, and modifications from drugs and environmental xenobiotics.

Stewart and associates (1976a) advocate the use of breath analysis to monitor DCM exposure because it is a noninvasive method and avoids the problems associated with the multiple blood sampling required for determinations of blood DCM and COHb levels. In addition, breath samples can be readily collected with little inconvenience in the immediate post-exposure period and also at later periods. Analysis of DCM in those samples by infrared spectroscopy or gas liquid chromatography provides both an identification and a measurement of the magnitude of exposure.

Stewart and his associates have constructed a "family" of post-exposure breath decay curves spanning 20 hours from controlled and known inhalation exposures of volunteers in the laboratory. The concentration of DCM in the alveolar air during and after exposure is directly related to the average inhalation exposure concentration. When the duration of exposure and work intensity are known, the total body burden can be estimated by reference to "standard" breath concentration curves. Because of large variations between individuals exposed under identical conditions, only very approximate DCM body doses can be estimated. The 2-hour period following exposure appears to be the most reliable breath sampling time for estimating the TWA DCM exposure concentration. Stewart et al. (1976a) provide little information on the statistical reliability or the inter-individual variation expected in the predictive values for exposure burdens, although they point out the desirability of constructing "individualized" breath decay curves for every person expected to be exposed to DCM. Peterson (1978), using the same experimental exposure data as Stewart et al. (1976a), has developed empirical equations relating post-exposure breath concentrations to exposure time, duration, and blood COHb levels.

4.7 SUMMARY AND CONCLUSIONS

The metabolism and pharmacokinetics of DCM have been extensively studied. At ambient temperature, DCM is a volatile liquid with high lipid solubility and modest solubility in water; therefore, the principal routes of entry to the body are by pulmonary and oral absorption. Comparatively fewer data are available on the metabolism and pharmacokinetics of absorption and excretion of DCM in humans, but these kinetics have been extensively studied in the rodent. Absorption from the GI tract is rapid and complete, occurring by first-order passive processes in the rat; the kinetics of peroral absorption and post-absorption disposition and elimination are influenced by the dosing vehicle. Pulmonary absorption also occurs by first-order diffusion processes in both humans and rats. There are three distinct components whose rate constants correspond to tissue loading of at least three major body compartments. At equilibrium with inspired air concentration, a blood/air partition coefficient of about 10.5 at 37°C has been observed.

Distribution of DCM in the tissues is consistent with its lipophilic nature and modest water solubility. The chemical readily crosses the blood-brain and placental barriers. Concentrations occurring in all major tissue organs are dose related to inspired air concentration or to oral dosage. During inhalation exposure, the quantity of DCM absorbed is also dependent on body weight and fat content of the body; the adipose tissue/ blood coefficient at 37°C is about 7 and is about 0.8 to 1.0 for brain and liver tissues. The rate of tissue loading with a given inspired air concentration is increased with physical activity and with exposure duration, with steady-state body equilibrium requiring more than 6 hours.

The kinetics of elimination of DCM from the body are complex and are dominated by two major and parallel processes: (1) pulmonary elimination of unchanged DCM, and (2) hepatic metabolism of DCM. Pulmonary elimination follows first-order kinetics, is independent of body dose, and exhibits at least three distinct body compartments with half-times for humans of about 8 to 23 minutes, 40 to 80 minutes, and 360 to 390 minutes, and half-times for rats of 1 to 2 minutes, 12 to 16 minutes, and 47 minutes, respectively. The longest half-time is associated with the lipid and adipose tissue body compartment. The differences between humans and rats for the half-times of pulmonary elimination from body compartments are presumed to be caused by differences in pulmonary and cardiovascular functions. Hepatic metabolism is body-dose dependent and saturable, and follows nonlinear Michaelis-Menten kinetics. Body dose, as given by oral dosing, also is subject to the kinetics of first-pass hepatic metabolism and first-pass pulmonary excretion. The estimated K_m for overall metabolism of DCM in the rat, as obtained from inhalation exposures, is about 400 ppm. This inhalation concentration for 6 hours produces an estimated body dose of approximately 50 mg/kg. For the body dose of 50 mg/kg, 70 percent of the DCM is metabolized, and for a 5.5 mg/kg body dose resulting from 50 ppm for a 6-hour exposure, 95 percent is metabolized. The inhalation concentration saturating overall metabolism of DCM is about 800 ppm ($2 \times K_m$).

The hepatic metabolism of DCM occurs by two enzymatic pathways: (1) an inducible cytochrome P_{450}-mediated microsomal oxidative dehalogenation to CO, and 2) a noninducible cytosolic glutathione transferase dehalogenation system yielding formaldehyde and formic acid, which are further metabolized to CO_2. The activities of these two pathways are approximately equal at low body burdens of DCM, and both pathways are saturable at high body burdens. Covalent binding to cellular macromolecules (proteins and lipids) by putative reactive intermediates of metabolism, formyl chloride from microsomal oxidative metabolism and S-chloromethyl glutathione and formaldehyde from cytosolic metabolism, has been shown to occur *in vitro* with rat hepatic microsome preparations and intact isolated rat hepatocytes and *in vivo* after ^{14}C-DCM injections to rats. No evidence was found in these studies for the occurrence of covalent binding to DNA.

The occurrence of increased blood COHb levels in humans and animals exposed to DCM is a consequence of hepatic microsomal oxidative metabolism of DCM to CO. The COHb produced is additive to COHb formed from exogenous CO. A functional relationship exists between the DCM inhalation concentration, duration of exposure, and the time course and peak blood COHb level. The blood COHb level achieved is determined by the nonlinear kinetics (Michaelis-Menten kinetics) of hepatic metabolism of DCM to CO and the parallel linear (first-order) kinetics of pulmonary elimination of CO from circulating COHb. Because hepatic metabolism of DCM to CO is saturable, the zero-order kinetics of CO production constrains blood COHb accumulation to an upper, limited concentration of 12 to 15 percent COHb, as observed experimentally. However, human studies have demonstrated that exposures to DCM at levels up to about 150 ppm (521 mg/m^3) are unlikely to exceed the biological TLV© for blood COHb (5 percent) recommended by the National Institute for Occupational Safety and Health (1976). Kinetic parameters for the production of CO and formation of COHb, calculated from experimental DCM exposure data of blood COHb concentrations in humans and rats, have similar values. Estimations of V_{max} for humans and rats are 15 and 13.5 percent COHb per 7-hour exposure, respectively, and estimates of K_m are 200 and 170 ppm, respectively. These findings indicate that the CO pathway is saturated with an inspired air concentration of approximately 400 ppm DCM (2 x K_m) in both humans and rats.

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5. HEALTH EFFECTS OF DICHLOROMETHANE

5.1 HUMAN HEALTH EFFECTS

5.1.1 Acute Exposures

5.1.1.1 Nervous System and Behavior--Virtually all experimental exposure studies of DCM using humans have studied acute exposures and neural or behavioral dependent variables. Using between three and eight subjects, Stewart et al. (1972b) reported that subjects had symptoms of "lightheadedness" and had difficulty with speech articulation at DCM levels higher than 868 ppm (3,012 mg/m^3) for more than 1 hour. They also reported anecdotal data about increased amplitude in certain visual evoked potentials at about the same exposure levels; however, no statistical analyses were possible because of the small number of subjects. In a later study, they reported that 50, 100, or 250 ppm (173, 347, or 867 mg/m^3) DCM had no effects on the Romberg equilibrium test, vigilance, coordination, or arithmetic skill (Stewart et al., 1973).

Winneke (1974) and Winneke and Fodor (1976) exposed female human subjects to 0, 300, 500, and 800 ppm (0, 1042, 1737, and 2,779 mg/m^3) DCM for up to 3 hours. They measured performance on a series of hand-eye coordination tasks including tapping pursuit rotor and hand steadiness. They also measured the critical flicker fusion (CFF) frequency and performance on an auditory signal detection task. Concentration-related decrements in signal detection, beginning at 300 ppm (1042 mg/m^3), were observed. CFF frequency also decreased in a concentration related fashion. Many motor decrements were observed at 800 ppm (2,779 mg/m^3), the only concentration at which these tests were conducted. Fodor and Winneke (1971) reported a similar study in which 300 and 800 ppm (1,042 and 2,779 mg/m^3) both decreased CFF frequency and impaired signal detection behavior; however, the two DCM levels did not produce effects significantly different from each other.

Gamberale et al. (1975) investigated the effect of DCM on psychomotor and cognitive performance in 14 men, ages 20 to 30 years, divided at random into two groups. The first group was initially exposed to progressively increased concentrations of DCM--250, 500, 748 and 997 ppm (869, 1,737, 2,599, and 3,464 mg/m^3)--through a face mask. Seven days later they were observed under control conditions. The second group was studied under identical conditions, but in reverse order. The subjects were exposed to each concentration for 30 minutes with no break in exposure; total exposure time for each individual was 2 hours.

At each concentration, a peak plateau of alveolar DCM occurred in about 10 minutes. Heart rate showed no change. During the two hours of exposure to DCM, no statistically significant impairment in any measured performance was observed, although reaction time appeared to be more irregular during exposure than during control conditions. Numerical ability and short-term memory were unaffected. The experiment did not consider any latency effects.

Putz et al. (1976) exposed six men and six women (ages 18 to 40 years) to 201 ppm (698 mg/m^3) of 99.5 percent pure DCM or to 7 ppm (8 mg/m^3) CO for 4 hours in an 8-m^3 chamber with unspecified dynamics. Alveolar concentrations were measured hourly, and peripheral blood from a finger was taken before and after exposure. Peak alveolar CO after 4 hours of DCM exposure was about 17 ppm (19.5 mg/m^3), and COHb was 5.1 percent. Alveolar CO after CO exposure for 4 hours was 4 ppm (4.6 mg/m^3), and COHb was 4.85 percent. Thus, there was COHb and alveolar CO equivalence between the two compounds. Eye and hand coordination at the end of the exposure period was depressed in subjects exposed to either compound, compared with controls, with DCM depression being greater than CO depression. The authors concluded that CO may have been primarily responsible for the decreases in performance after exposure to both compounds.

These studies can only be considered a preliminary survey of possible CNS and behavioral consequences of DCM exposure. Only one study (Winneke, 1974) reported concentration effects data on CFF and signal detection, both possibly involving the alertness of the subjects. CFF is a primitive measure of CNS activation. The lowest concentration of DCM reported to have affected behavior was 200 ppm (695 mg/m^3) (Putz et al., 1976). They also reported vigilance decrements in addition to compensatory tracking decrements. In spite of these few preliminary findings, the results are consistent with the hypothesis that DCM effects are depressant and also agree well with the available laboratory animal data, reported elsewhere in this chapter.

Data are required on the effects of DCM upon other behaviors. No data were found regarding cognitive skills, and no concentration effects data were available for motor skills or hand-eye coordination. No quantitative tests of electrophysiological effects of DCM in humans were found. Finally, no data regarding the effects of DCM in combination with other pollutants or medicinals were found.

Putz et al. (1976) concluded that DCM produced its effects via its principal metabolite, COHb. Stewart et al. (1972a) had suggested this possibility.

Winneke (1974) presented evidence suggesting that DCM is more deleterious than equivalent levels of CO. He compared subjects exposed to 50 or 100 ppm CO for 5 hours to subjects exposed to 300, 500, and 800 ppm (1042, 1737, and 2779 mg/m^3) DCM for 4 hours. DCM-exposed subjects showed more effects on the same tasks. Comparison with data presented by Putz et al. (1976) reveals that equivalent COHb levels were produced by 70 ppm CO and 200 ppm (695 mg/m^3) DCM. Examination of the behavioral results of the study by Putz et al. reveals that DCM always produced greater decrements than did CO. Logically DCM should be more toxic than CO, since it not only produces COHb, but, because of its lipophilic qualities, it probably has some primary neural effect. Additional data are required to explore this issue.

5.1.1.2 Other Experimental DCM Effects--Stewart et al. (1972a,b) reported no effects of exposure to 500 or 1,000 ppm (1,737 or 3,474 mg/m^3) DCM for 2 hours on various hematological, hepatic, or renal measures. Continuous electrocardiographic monitoring during one or two exposures to 1000 ppm (3,474 mg/m^3) was not reported to cause arrhythmias. Gamberale et al. (1975) reported that DCM exposure up to 1,000 ppm (3,474 mg/m^3) produced no alterations in heart rate. Based on laboratory animal exposures, it is not reasonable to expect other internal organ system involvement in healthy humans with low-level acute exposures.

Stewart and Dodd (1964) studied groups of three to five men and women (25 to 62 years old) who immersed their thumbs in various chlorinated aliphatic solvents. Within the first 2 minutes of immersion in DCM, all subjects reported an intense burning sensation on the dorsal surface of their thumbs. Within 10 minutes, a feeling of coldness or numbness, alternating with the burning sensation, was reported; the slightest movement of the thumb triggered waves of searing and excruciating pain. Following removal from the solvent, their thumbs were described as "numb and cold" or "asleep." A very mild erythema and a hint of white scaling were the only overt signs of irritation. Within 1 hour, both the erythema and paresthesia subsided.

Studies of human blood in vitro from individuals homozygous for sickle cell anemia (Matthews et al., 1977, 1978) have shown that DCM caused hemolysis, preferentially of sickle and other abnormal cell types. These reports suggest that acute exposure to relatively high concentrations of DCM may result in anemia, particularly in individuals who are predisposed to hemolytic anemias, e.g., persons with sickle cell anemia or glucose-6-phosphate dehydrogenase deficiency. However, more compelling evidence is required to ascertain that such an effect exists.

5.1.1.3 Accidental Exposure--A number of case reports have implicated exposure to exceedingly high levels of DCM as one factor in human fatalities (Stewart and Hake, 1976; Kuzelova et al., 1975; Moskowitz and Shapiro, 1952; Bonventre et al., 1977; Winneke et al., 1981). However, systematic conclusions from accidental exposure data are difficult. Exact exposure levels and durations are unknown. Simultaneous exposure to other substances is often the case. Serious disturbances of the CNS and cardiovascular system in accidental overexposure situations have not been reported in any human case report, even following fatal exposures. The only evidence of human nephrotoxicity resulting from DCM overexposure was a finding of congested kidneys following a fatal exposure. Ocular toxicity other than eye irritation and congested conjunctivae has not been reported in humans exposed to DCM.

5.1.1.4 Occupational Exposure--Ott et al. (1983a,b,c,d,e) in a series of studies examined various health evaluations of employees occupationally exposed to DCM. This involved a mortality study, clinical laboratory evaluation study, metabolism data and oxygen half-saturation pressure study, and a 24-hr electrocardiographic monitoring study. The mortality study examines general mortality, ischemic heart disease, mortality, and cancer mortality, and is discussed in length in Section 5.3.3.2.2 as part of the section on evaluation of the carcinogenicity of DCM. No mortality effects attributable to DCM exposure were demonstrated in the study, but given qualifications of low statistical power and confounding factors, these results do not exclude the possibility of increased health risks in the study population.

Ott et al. (1983a,c) studied six serum constituents that had the potential to detect possible liver injury, in relation to DCM exposures that ranged from 60 to 475 ppm (208 to 1650 mg/m^3). The exposed group consisted of 313 individuals; the reference group consisted of 321. A dose-related rise in serum bilirubin was observed for both men and women. A consistent positive association between total bilirubin and DCM exposure was found in three of four subgroups by exposure. The meaning of this and the sensitivity of changes and possible associated liver damage are not clear and tests which could clarify the situation (direct bilirubin) were not performed. Women in the subgroup exposed to 475 ppm (1650 mg/m^3) DCM showed an increase in red cell counts, hemoglobin, and hematocrit, but men did not. These findings are suggestive of a hematopoietic effect. As expected, the carboxyhemoglobin concentration showed an increase and a possible association with DCM exposure for all four subgroup exposures. Two standard liver function tests were not performed (SGOT, SGPT), and these might have proved to be more sensitive indicators of liver function.

Twenty-four-hour electrocardiographic monitoring was conducted by Ott et al. (1983d) in a study involving 50 employees, 24 of whom were occupationally exposed to DCM. All subjects were white males between the ages of 37 and 63 years. The results showed neither an increase in ventricular or supraventricular ectopic activity nor episodic ST-segment depression to be associated with exposure to DCM ranging from a time-weighted average DCM exposure of 60 to 475 ppm (208 to 1650 mg/m^3). This study may have lacked adequate power to detect expected changes. Other critical factors that may limit the meaning of the study include choice of subject, exercise regimen, and control possible in laboratory studies but difficult for field studies.

A further study by Ott et al. (1983) of 136 DCM-exposed and 132 non-exposed employees produced a calculated decrease in oxygen half-saturation pressure between 2 and 4 mm Hg among persons previously exposed to $\geq$ 300 ppm DCM for an 8-hour work day. The expected dose-response increase, in the level of carboxyhemoglobin and carbon monoxide, were produced from DCM exposure. Physiological adaptations may have occurred, but risk of ischemic events was not demonstrated in the associated Ott et al. (1983b) study on mortality, discussed in more detail in Section 5.3.3.2.2.

5.1.2 Chronic Effects

5.1.2.1 Experimental Exposure--Stewart and his colleagues extended their studies of the effects of DCM on human subjects to include longer exposure. In one study (Hake et al., 1974), male volunteers were exposed 5 days/week for 5 weeks to DCM concentrations of 50 ppm (174 mg/m^3) in week 1, 250 ppm (868 mg/m^3) in week 2, 250 ppm (868 mg/m^3) in week 3, 100 ppm (347 mg/m^3) in week 4, and 500 ppm (1730 mg/m^3) in week 5. Three subjects were exposed for 1 hr/day, three subjects were exposed for 3 hr/day, and four subjects were exposed for 7.5 hr/day. This complex experimental design was further confounded by attempts to separate smoking and nonsmoking populations. The information that can be gathered from this report is summarized in Table 5-1. Alongside is a summary of a companion report from Stewart's group (Forster et al., 1974), which exposed nine female subjects to 250 ppm (868 mg/m^3) DCM for 1, 3, or 6.5 hr/day for 5 days.

TABLE 5-1. COHb CONCENTRATIONS IN NONSMOKERS EXPOSED TO DCM AT 250 ppm (869 mg/m^3) FOR 5 DAYS

Exposure Time hr/day	Mean of Daily Maximum Observed Average COHb (%)	
	Male (n)[a]	Female (n)[b]
Pre-exposure	0.9 (5)	1.4 (8)
1	3.3 (1)	3.5 (3)
3	7.0 (1)	5.1 (1)
7.5	9.6 (3)	10.1 (4)

[a] Hake et al., 1974.

[b] Forster et al., 1974.

Carboxyhemoglobin levels up to 10 percent were reported in 10 men (20 to 39 years old) and 9 women (20 to 41 years old) exposed to DCM concentrations between 40 and 500 ppm (139 and 1,737 mg/m^3) by inhalation (Peterson, 1978). Exposure durations were between 1 hr and 7.5 hr/day for not more than 5 successive days.

5.1.2.2 Occupational Exposure--Toxic encephalosis due to occupational exposure to DCM was diagnosed in a chemist (Weiss, 1967). Other etiologies, including exposure to other chemicals, were not considered. No liver or kidney damage or ECG alterations were reported. Erythema and fissures appeared on the hands and forearms.

Collier (1936) reported four cases of occupational exposure to paint remover containing approximately 96 percent DCM. The men, all of whom were professional painters, had been exposed to lead for 5 to 14 years. During one autumn, while they were removing paint, the workers complained of loss of appetite, dullness, faintness, and giddiness while using the remover and during the following few hours. The author diagnosed the symptoms as resulting from slight chronic lead intoxication and acute DCM-induced toxemia.

Barrowcliff (1978) and Barrowcliff and Knell (1979) reported that an individual exposed to 300 to 1,000 ppm (1,032 to 3,474 mg/m^3) DCM for 3 years developed bilateral temporal lobe degeneration. This was thought to result from chronic CO intoxication as a result of exposure to DCM.

5.2 EFFECTS ON LABORATORY ANIMALS

Much of the experimental research on the effects of DCM has been done on laboratory animals. Such work can be used to elucidate the general principles of DCM action and to discover which target organs or systems are involved. Generalization of laboratory animal findings to humans, however, is not straightforward. Human health effects must, in the final analysis, be assessed in man.

5.2.1 Acute Effects

5.2.1.1 Lethality--Only preliminary work has been done on the lethality of DCM. No systematic data exist which would make it possible to specify a time-by-dose-by-lethality function.

Table 5-2 is a summary of lethality data. The inhalation data are remarkably consistent between rats and mice. The one report on guinea pigs suggests that they may be more sensitive to DCM but procedural variation cannot be ruled out. Data on shorter exposures are required since accidental exposures and substance abuse frequently involve high concentration, short-term profiles. A curve of LC_{50} for a range of exposure times would be valuable.

Oral and intraperitoneal injection LD_{50} data appear to be remarkably consistent across investigations and species. Exceptionally low values (Zakhari, 1977) and high values (Ugazio et al., 1973) are possibly due to procedural differences. The mean LD_{50} from Table 5-2, excluding the low and high values mentioned, for injection and oral doses in rodents is near 2000 mg/kg. No data for other sites of injection were found. Oral and intraperitoneal data cannot be compared with inhalation data because of the "first pass effect" which occurs following oral or intraperitoneal administration.

Morbidity resulting after short-term exposure includes effects on several different organs. Ocular damage, liver damage, kidney damage, changes in cardiac parameters, and increased pancreatic bile duct flow all occur.

The course of events during lethal exposures to DCM via inhalation was described by Berger and Fodor (1968) who exposed rats to DCM concentrations ranging from 2,800 to 28,000 ppm (9.7 to 97.3 g/m^3). In these exposures an initial period of excitation was followed by a deep narcosis accompanied by a decrease in muscle tone and a reduction in brain electrical activity. Rats exposed to concentrations of 25,000 to 28,000 ppm (86.9 to 97.3 g/m^3) ceased to exhibit electroencephalographic (EEG) activity after only 1.5 hours; those

TABLE 5-2. ACUTE LETHAL TOXICITY OF DCM

Route of Administration	Species	Dose	Duration of Exposure	Effect	Reference
Oral	mice	1987 mg/kg	-	LD_{50}	Zakhari (1977)
	rats	4368 mg/kg	-	LD_{50}	Ugazio et al. (1973)
	rats	2388 mg/kg	-	LD_{50}	Kimura et al. (1971)
Intraperitoneal	mice	448 mg/kg	-	LD_{50}	Zakhari (1977)
	mice	1330 mg/kg	-	100% survival	Plaa and Larson (1965)
	mice	1995 mg/kg	-	20% survival	Plaa and Larson (1965)
	mice	1990 mg/kg	-	LD_{50}	Klaassen and Plaa (1966)
	mice	1990 mg/kg	-	LD_{50}	Gradiski et al. (1974)
	dogs	1260 mg/kg	-	LD_{50}	Klaassen and Plaa (1967)
Inhalation	mice	14,100 ppm	6 hours	LD_{50}	Gradiski et al. (1974)
	mice	16,100 ppm	7 hours	LD_{50}	Svirbely et al. (1947)
	rats	25,600-28,000 ppm	1.5 hours	(a)	Berger and Fodor (1968)
	rats	16,100-18,150 ppm	6 hours	(a)	Berger and Fodor (1968)
	guinea pigs	11,500 ppm	6 hours	LD_{50}	Balmer et al. (1976)

(a)Cessation of brain electrical activity in all animals. 1 ppm = 3.474 mg/m^3.

exposed to concentrations between 16,000 and 18,000 ppm (55.6 and 62.5 g/m^3) ceased to exhibit EEG activity after 6 hours.

5.2.1.2 Nervous System and Behavior--The nervous system is a likely target organ because DCM is lipophilic and thus is concentrated in myelin. Rat brain tissue has been shown to have high concentrations of DCM following exposure (Savolainen et al., 1977; Bergman, 1978; see also Section 4.1.3). Pankow et al. (1979) showed that nervous tissue function is affected by DCM dosage. They demonstrated a linear decrease in sciatic nerve conduction velocity, using intraperitoneal injections of DCM at dose levels of 1 to 6 mmoles/kg.

EEG and rapid eye movement (REM) in rats have been used to characterize sleep during DCM inhalation. These data form a concentration-related continuum from 500 to 3000 ppm (1,737 to 10,422 mg/m^3) in which REM sleep was reduced in duration beginning at about 500 ppm (1,735 mg/m^3) (Fodor and Winneke, 1971). At 3,000 to 9,000 ppm (10,410 to 31,230 mg/m^3), sleeping time was increased with a further decrease in REM sleep (Berger and Fodor, 1968). Higher concentrations eventually produced a flat EEG after coma (Berger and Fodor, 1968). The above may be viewed as a continuum of effects beginning with REM sleep reduction at 500 ppm (1,737 mg/m^3) and ending in brain death after 1.5 hours at 27,000 ppm (93.8 mg/m^3) or 6 hours at 17,000 ppm (59 mg/m^3).

Measures of general activity level are commonly used to characterize behavioral effects. However, general activity is not a simple or unidimensional measure. Frequently, some measures of activity level are affected though others are not. A concentration of 5,000 ppm (17,370 mg/m^3) DCM administered for 1 hour to male rats was sufficient to decrease running activity (Heppel and Neal, 1944). Running activity increased after exposure, but was lower than activity in the same male rats when they had not been exposed to DCM. Similarly, Thomas et al. (1971) reported that the spontaneous activity of mice was decreased by a 3-hour exposure to 1,000 ppm (3,474 mg/m^3) of DCM. Weinstein et al. (1972) exposed female mice in groups to about 5,000 ppm (17,370 mg/m^3) DCM for 24 hours and observed progressive decreased spontaneous activity.

In an experiment designed to determine whether the passive-avoidance conditioning task was appropriate for measuring impairment of learning ability in mice, Alexeff and Kilgore (1983) exposed groups of Swiss-Webster mice (male) to 168 mg/l DCM until loss of righting reflex ($\sim$ 20 sec). Groups of 10 mice in age ranges of 3, 5, and 8 weeks were tested in the task described by Essman and Alpern (1964) and modified by Reiter et al. (1973). Recall of the conditioning task was tested from 1 to 4 days following exposure. In all three age groups, the proportion of animals that learned the task was consistently less in the groups exposed to DCM. The 3-week-old mice exhibited the most consistent impairment in learning. When recall latencies of this age group were compared to latencies of the other groups by the Kruskal-Wallis test, recall was significantly lower ($p = 0.01$). Learning impairment was observed particularly in those animals tested at 1 or 2 days following training. The percentage of mice in the control group that recalled the task was significantly higher than the percentage of DCM-exposed mice ($p = 0.024$). The learning impairment observed was not reported by the authors to be related to differences in observable behavior, motor activity, or analgesia. In the analgesia test, morphine was used as a positive control.

Little can be said about the effects of acute DCM exposure upon the nervous system and behavior of laboratory animals. The effect appears to be consistently depressive above 500 ppm (1,737 mg/m^3). However, to say that an effect is "depressive" is to oversimplify matters. Other than the report by Alexeeff and Kilgore (1983), no work has been found on the effects of acute DCM exposure upon motor tasks, sensory discrimination, schedule-controlled behavior, or response acquisition in laboratory animals. Similarly, no research has been published concerning the effects of acute DCM exposure on any electrophysiological variable other than nerve conduction velocity, and secondarily, brain electrical activity. There have also been no follow-up studies to show whether acute exposure effects were (or were not) reversible.

5.2.1.3 Cardiovascular Effects--The use of DCM in the study of cardiovascular effects generally has been at a very high level (> 5,000 ppm; 17,000 mg/m^3) in short-term exposures (≦ 5 minutes). While knowledge of effects of such levels would be important for accidental or substance abuse exposures, they are not otherwise particularly useful for assessment of health concerns relating to environmental exposures. A summary of the effects of high level exposure in rabbits and dogs is shown in Table 5-3 and draws upon the work of Taylor et al. (1976) and Aviado et al. (1979).

Several reports here indicated that exposure of animals to levels of DCM exceeding 5,000 ppm (17,000 mg/m^3) caused cardiac arrhythmias (Aviado, 1975; Aviado, 1977; Zakhari, 1977; Belej et al., 1974; Aviado and Belej, 1974). Additional cardiovascular effects of exposure to exceedingly high levels of DCM were reported by Loyke (1973), Douglas et al. (1976), Wilkinson et al. (1977), Adams and Erickson (1976), and Pryor et al. (1978).

TABLE 5-3. SUMMARY OF CARDIOTOXIC ACTION OF 5% DCM

Function[a]	Taylor et al. (1976)	Aviado et al. (1977)[b]
HR	1% ↑ (NS)	11% ↓ (NS)
MAP	0%	4% ↓ (NS)
LVP	0%	4% ↓ (NS)
CVP	0%	---
LVEDP	9% ↑ (NS)	125% ↑
LVdP/dt	17% ↓	22% ↓ (NS)
CO	14% ↓	36% ↓
SV	14% ↓	33% ↓
VR	25% ↑	44% ↑
Comments:	Rabbits	Dogs
	Pentobarbital-anesthetized Spontaneously breathing Close-chested 1-min. exposure (except 1.5 min. for CO) Static dose (5%)	Pentobarbital-anesthetized Artificially ventilated Open-chested 5-min. exposure Increasing doses (0.5, 1.0, 2.5, and 5.0%)

[a] Key:

HR - Heart Rate
MAP - Mean Arterial Pressure
LVP - Left Ventricular Pressure
CVP - Central Venous Pressure
LVEDP - Left Ventricular End-Diastolic Pressure
LVdP/dt - Left Ventricular Rate of Time-Dependent Pressure Change
CO - Cardiac Output
SV - Stroke Volume
VR - Vascular Resistance (Peripheral or Systemic)
NS - Not significantly different from control values at $p < 0.05$

[b] Results presented are for the highest dose (5%) of dichloromethane.

5.2.1.4 Hepatic, Pancreatic, and Renal Effects--Klaassen and Plaa (1966) injected Swiss-Webster mice (25 to 35 g) intraperitoneally with analytical grade DCM. Doses of 13,300 mg/kg had no effect on Bromsulphalein (BSP) retention or on serum glutamic pyruvic transaminase (SGPT) activity 24 hours after injection. No histopathological change was seen upon examination of the liver.

In the dog, however, effects on the liver by DCM were reported by Klaassen and Plaa (1967). They derived an ED_{50} (effective dose in 50% of animals) for SGPT elevation of 798 mg/kg (the LD_{50} was 1,260 mg/kg). Histopathology showed moderate neutrophilic infiltration in the sinusoids and portal areas. Necrosis was not seen. At "near lethal" doses, there was vacuolization of centrilobular hepatocytes.

Reynolds and Yee (1967) gavaged fasted male Charles River rats (100 to 300 g) with doses of ^{14}C-DCM up to 2,210 mg/kg dissolved in mineral oil. The rats were sacrified 24 hours later. No liver necrosis was seen and no change in glucose-6-phosphatase activity was observed. Labelled DCM (^{14}C) was found in hepatic lipids and proteins, minimally in the lipids and in high concentrations in proteins. Similar patterns of incorporation were found in liver microsomes.

Weinstein et al. (1972) exposed female ICR mice (13 to 20 per group, 23 to 27 g) continuously to approximately 4,893 ppm (17,000 mg/m^3) vaporized technical grade DCM in Thomas Domes, at pressures slightly below ambient. Temperature, humidity, CO_2, and air flow were monitored and controlled. During a 24-hour period, there was a progressive decrease of spontaneous activity. Body weight decreased, liver weight increased absolutely and as a ratio to body weight, liver triglycerides increased (indicating liver toxicity), glycogen decreased, and protein synthesis was reduced as shown by reduction of ^{3}H-leucine incorporation.

Many histological changes appeared. Fatty infiltration after 24 hours involved the entire lobule; centrilobular hepatocyte nuclei became smaller and denser, and ballooning developed. Staining showed glycogen reduction. Progressive changes began after 12 hours of exposure. Smooth (SER) and rough (RER) endoplasmic reticulum showed changes: polysomes broke down, ribosomal particles detached from RER in the centrilobular cells, RER membranes broke up into vesicles, perinuclear cisternae dilated, and lipid droplets increased. Mitochondria, however, were unaffected. The authors concluded that the pattern of liver damage was similar to that following carbon tetrachloride exposure.

Morris et al. (1979) exposed male Hartley guinea pigs weighing 500 to 750 g for 6 hours to a 5,181 ppm (18,000 mg/m^3) vapor concentration of reagent grade DCM. Animals were sacrificed immediately after exposure and liver samples were taken. Liver and serum were analyzed for triglycerides, which increased markedly in the former and decreased in the latter. Liver phospholipids showed no change, neither did serum-free fatty acid. Liver slices were incubated with ^{14}C-palmitic acid. The uptake of ^{14}C was similar to controls. Uptake of ^{14}C-leucine did not differ between controls and exposed samples.

Differences in protein synthesis in the above two studies may be due to different periods of exposure and the use of different animal species.

Harms et al. (1976) cannulated the bile duct of male Sprague-Dawley rats (350 to 450 g) after pretreatment with intraperitoneal injections of 670 mg/kg DCM dissolved in corn oil. Tritiated ^{3}H-insulin was instilled into the duct 24 hours later. DCM seemed to induce an increase in pancreatic bile duct flow, which was unrelated to any observed hepatic effect. Hamada and Peterson (1977), in a follow-up study, intraperitoneally injected 860 mg/kg DCM (dissolved in corn oil) in male Sprague-Dawley rats (280 to 320 g). The bile duct was cannulated, and bile duct pancreatic flow and its contents were measured and compared with controls. DCM induced increased pancreatic bile duct flow, decreased protein concentration, and increased chloride, sodium, and potassium. Bicarbonate was unaffected. There was no statistically significant difference in wet weight of the pancreas or in total bile flow; this may indicate a reduced hepatic bile flow. An experiment with secretin indicated that these changes were not related to that substance or, after an ancillary experiment with atropine, to any cholinergic effect.

Plaa and Larson (1965) injected 10 male Swiss mice (18 to 30 g) intraperitoneally with 1330 mg/kg of DCM (source and quality unidentified) dissolved in corn oil. No glycosuria or proteinuria was detected 24 hours later. Two surviving mice of 10 injected with 1,995 mg/kg DCM had proteinuria but not glycosuria. No renal histopathology was seen after the lower dose. Proteinuria following the higher dose suggested that there might have been some tubular damage.

Klaassen and Plaa (1966) also injected male Swiss-Webster mice (25 to 35 g) intraperitoneally with 13.3 g/kg analytical grade DCM. These mice showed no glycosuria, proteinuria, or changes in BSP excretion 24 hours after injection. Two groups of 10 mice each were gavaged for 3 days with 5 g/kg 60 percent ethanol and equicaloric solutions of dextrose. All animals were then injected with DCM, and urine was collected and analyzed 24 hours later. BSP excretion remained within control limits, indicating the absence of a renal lesion, but the authors reported, "A few kidneys exhibited hydropic degeneration with minimal necrosis of the convoluted tubules."

Kluwe et al. (1982) have investigated the effects of DCM on renal tubular cells of adult male Fischer 344 rats by injecting DCM (1300 mg/kg), i.p., in corn oil. Renal proximal tubular swelling was observed in the cortex and in the outer medulla. Tubular cell functions measured included organic ion transport, ability to maintain a potassium gradient, and the rate of oxygen utilization. None of these functions was altered by DCM. Rats were sacrificed 2, 12, 24, 48, or 96 hours after injection, the kidneys were removed, and thin slices were prepared. DCM was not observed to alter glomerular, distal tubular, inner medullary or papillary cell morphology. Although DCM did cause an increase in the fraction of water in renal tissue, the lack of effect on other parameters suggested to the authors that little or no functional cell disturbance had occurred.

5.2.1.5 Other effects-- Ballantyne et al. (1976) studied the effects of liquid DCM on various ocular parameters by instilling 0.1 and 0.01 ml of DCM in rabbits' eyes. Lachrymation persisted for a week, inflammation of the lids and conjunctivae for 2 weeks, conjunctival edema for a week, sloughing for 3 days, and increased corneal thickness for 9 days. Iritis and keratitis appeared within 6 hours and lasted for 7 and 14 days, respectively. Intraocular pressures increased also. Increased corneal thickness developed in rabbits exposed to DCM vapor at concentrations of 504 and 5,040 ppm (1,750 and 17,500 mg/m^3).

Sahu and Lowther (1981) observed that inhalation of DCM by 2-month-old Sprague-Dawley rats led to pulmonary injury, presumably rupture of type II alveolar cell membranes and release of cell contents into the airways. Rats were exposed to about 4000 ppm (13,896 mg/m^3) for 5 hr/day, 5 days/wk, for 4 weeks. Pulmonary secretions were obtained by lung lavage. The soluble supernatant of the lung homogenate and the cell-free lung lavage were used for determination of lung lipid peroxidation. Lipid peroxidation was significantly elevated ($P < 0.05$).

5.2.1.6 Summary of Effects of Acute Exposure on Laboratory Animals--Of all the organ systems studied, the central nervous system appears to be affected by DCM at levels ranging from 500 to 1,000 ppm (1,737 to 3,474 mg/m^3). While only short-term exposures were used, levels of over 20,000 ppm (69,000 mg/m^3) were required before cardiac function changes were produced. Hepatic effects were reported at exposure levels as low as 5,000 ppm (17,370 mg/m^3). While it is difficult to compare results of injection studies to those of inhalation exposures, it appears that half the LD$_{50}$ is required to produce hepatic or renal changes. The above comparisons are based upon the lowest level reported. In two cases, however, the behavioral data were obtained for levels of exposure at or below 1,000 ppm. The weight-of-evidence for short-term exposures indicates that the CNS is the primary target organ for DCM.

The concentrations of DCM necessary to depress cardiac function (25,000 to 50,000 ppm) in acute experiments are so high that chronic long-term exposures of humans to levels considerably in excess of 250 ppm (865 mg/m^3) would be unlikely to have any effect. However, comparatively little research has been done on acute DCM effects on laboratory animals.

5.2.2 Chronic Effects

5.2.2.1 CNS Effects--In early studies of DCM chronic exposure (Heppel et al., 1944; Heppel and Neal, 1944), dogs, monkeys, rabbits, guinea pigs, and rats were exposed 4 hours/day, 5 days/week for 8 weeks to 5,000 or 10,000 ppm (17,370 or 34,740 mg/m^3) DCM. Unfortunately, only qualitative observations of behavioral "symptoms" were reported. No symptoms were noted in groups exposed to 5,000 ppm (17,300 mg/m^3). At 10,000 ppm (34,740 mg/m^3) all species were affected but in different ways. Dogs became excitable and hyperactive. Monkeys became progressively more inactive and, by the end of each daily exposure, lay prostrate with barely perceptible respiration. Rabbits first were excitable and then became inactive toward the end of each daily session. Guinea pigs and rats simply became more inactive. All species appeared well within 1 hour after cessation of exposure. Only monkeys (n = 2) appeared to develop behavioral tolerance. No other cumulative effects were reported over the course of the 8 weeks.

Weinstein et al. (1972) exposed female mice to 5,000 ppm (17,300 mg/m^3) DCM continuously for 7 days. Only qualitative behavioral observations were reported. For the first few hours of exposure, the animals exhibited an increased activity level and increased food and water intake. This was followed by decreased activity, "hunched" posture, dehydration, and the appearance of roughened yellow coat. By the fourth day of exposure, many of the mice had adapted so that by the end of the study they were virtually normal.

Thomas et al. (1972) studied activity level in mice continuously exposed to 25, 100, or 1,000 ppm (87, 347, or 3,474 mg/m^3) for 14 weeks. The lowest exposure level elevated activity level. There was no effect at 100 ppm (347 mg/m^3); at 1,000 ppm (3474 mg/m^3), there was reduced activity level. The increased activity level in the 25 ppm (87 mg/m^3) exposed group was, if replicable, probably due to the increased sensory stimulation due to the odor of DCM.

Savolainen et al. (1977) exposed male rats to 500 ppm (1,737 mg/m^3) DCM, 6 hr/day for 4 days. Exposed rats engaged in more grooming behavior than controls during the first exposure hour but by the seventeenth exposure hour (third day) exposed rats no longer differed from controls. Other behaviors were observed, but by their absence in the results summary, it may be assumed that only grooming was significantly affected. Biochemical analysis of tissue from the right cerebral hemisphere showed no difference in protein, RNA, or glutathione levels as compared with levels in control animals. Relatively small increases in acid proteinase and nonspecific cholinesterase activities were reported, but the determinations for treated animals were made by only two assays and may therefore be of questionable significance.

Since only one study of activity level is available, it is difficult to conclude much about neurobehavioral effects of chronic exposures. As in acute exposure studies, no other behaviors, such as sensory disturbance, motor response acquisition, or schedule-controlled behaviors, were studied. Where effects were seen, no follow-up studies were conducted to determine the irreversibility of effects.

Suggestions of species differences in DCM sensitivity occurred in the studies by Heppel et al. (1944) and Heppel and Neal (1944). Based on these qualitative observations, one could conjecture that DCM behaves in a manner similar to other anesthetics: There is an early excitatory phase followed by progressive depression. If some species had different sensitivities to DCM, they might remain in the excitatory phase or progress more rapidly into depression.

Several reports mentioned an increased tolerance to DCM over the duration of chronic exposures (Weinstein et al., 1972; Savolainen et al., 1977). Heppel et al. (1944) also reported monkeys to have adapted somewhat. Probably adaptation, like sensitivity, is a function of species, exposure level, and duration.

Because of the paucity of data and the qualitative approaches used, these conclusions must be regarded as tentative. CNS effects have been reported at exposure levels as low as 1,000 ppm (3,474 mg/m^3) but not lower. The one case of increased activity level at 25 ppm (87 mg/m^3) must be discounted.

5.2.2.2 Hepatic and Renal Effects--Many of the "high-dose" chronic animal studies with DCM have revealed a certain degree of liver and kidney involvement as target organs. The magnitude of this involvement increases in such a way that extremely high doses of DCM (depending upon dosage and duration of exposure) can produce toxic effects upon these organs. Heppel et al. (1944) observed moderate centrilobular congestion and fatty degeneration of the liver in dogs and guinea pigs exposed to 10,000 ppm (34,740 mg/m^3), 4 hours/day, 5 days/wk for 8 weeks. Weinstein et al. (1972) reported identical findings after exposing mice to 5,000 ppm (17,370 mg/m^3) continuously for 7 days. High mortality was observed by MacEwen et al. (1972) where 14 weeks of continuous exposures at 1,000 or 5,000 ppm (3,474 or 17,370 mg/m^3) resulted in severe toxic effects and a high degree of mortality in mice, rats, dogs, and monkeys.

More important, chronic mouse studies by Weinstein and Diamond (1972) and Haun et al. (1972) have revealed that continuous exposure even to levels of DCM as low as 100 ppm (347 mg/m^3) can effect changes in both liver function and cell architecture.

Weinstein and Diamond (1972) exposed ICR mice (17 to 25 g) continuously for 3 days to 10 weeks to 100 ppm (347 mg/m^3) of chemical grade DCM in a Thomas Dome with specified dynamic characteristics and at 96.66 Pa (725 mm Hg) pressure. Twelve groups of 16 mice each were used. Except for one instance at week 2, body weights were comparable to controls. Liver weights followed body weights, and in all cases, liver to body weight ratios were within control limits. Triglycerides increased approximately threefold at week 2, and nearly fourfold by week 3, but then decreased to about double at week 4. Four mice withdrawn at 3 days showed no abnormalities, but at 7 days, centrilobular fat accumulation was seen, accompanied by a decrease in liver glycogen. These abnormalities persisted to the termination of the experiment at 10 weeks. During this time, the nuclei enlarged. No other histopathology was seen under the light microscope. Under the electron microscope, autophagic vacuoles containing debris appeared in the hepatocytes. The smooth and rough endoplasmic reticulum showed no changes.

Haun et al. (1972) exposed mice, rats, dogs, and monkeys to 25 and 100 ppm (87 and 347 mg/m^3) reagent grade DCM for continuous exposure periods up to 100 days in Thomas Domes at ambient pressure. This is the longest continuous exposure study reported. Strain, sex, and size of the test animals were not specified. Exposure to the lower concentration had no observable effect, but exposure to the higher concentration resulted in positive fat stains and vacuolization. The rats showed nonspecific tubular degeneration and regeneration in the kidneys, but no changes in organ-body weight ratios.

Norpoth et al. (1974) tested DCM in studying the possibility of enzyme induction in inhalation of hydrocarbon solvents. Male SPF Wistar rats (80 to 100 g) were exposed 5 hours/day to vapors containing 0, 500, or 5,000 ppm (0, 1,737, or 17,370 mg/m^3 DCM) of the hydrocarbon solvents for 10 days or 250 ppm (869 mg/m^3 DCM) of the solvents for 28 days. There were 50 animals in the control group and six animals in each of the exposed groups. At the end of these exposure periods, the animals were killed and the concentration of liver cytochrome P_{450} and microsomal aminopyrine N-demethylase activity were determined.

Following the 10-day exposure, a significant increase in liver cytochrome P_{450} in animals exposed to 500 ppm (1,737 mg/m^3) but not in animals exposed to 5,000 ppm (17,370 mg/m^3), of the compound was reported. In contrast, aminopyrine N-demethylase activity was not elevated in animals exposed to 500 ppm (1,737 mg/m^3) DCM but was substantially elevated in those exposed to 5,000 ppm (17,370 mg/m^3) DCM. After 28 days of exposure to DCM at 250 ppm (868 mg/m^3), no changes were noted in liver enzymes, liver weight, or histologic appearance.

These results indicate that differential enzymatic induction can be produced by exposure to DCM. The inverse dose-response relationship noted for cytochrome P_{450} may be due to the combined effect of DCM and CO. Acclimatization may have occurred by the 28th day of exposure to ameliorate the effects seen at 10 days. In addition, the doses given in the second exposure period (28 days) were much smaller than during the first period.

Loyke (1973) induced chronic renal hypertension in 13 Sprague-Dawley rats (100 g, sex not specified). Both poles of one kidney were ligated and the contralateral one was removed. After maintenance of high systolic pressure for 3 months, 11 experimental rats and 3 controls were injected subcutaneously with 2 mg/kg unspecified DCM biweekly for 15 doses. Two hypertensive rats were used for positive controls and 11 normotensive rats as naive controls. DCM reduced the systolic blood pressure of the hypertensive rats from 200 to 160 mm Hg. The positive controls remained hypertensive. DCM was ineffective in reducing blood pressure in normotensive rats. No changes ascribable to DCM were seen in the liver.

In the Dow Chemical Co. (1980) chronic inhalation study in rats, exposure to 500, 1500, or 3500 ppm (1,737, 5,211, or 12,159 mg/m^3), for 6 hours/day, 5 days/ week for 2 years resulted in exposure-related non-neoplastic hepatic lesions in both males and females. Grossly, the effect was most prominent in females exposed to 3500 ppm and consisted of increased numbers of dark or pale foci. The percentages of total rats with any degree of vacuolization were 17 percent, 38 percent, 45 percent, and 54 percent in the males of the 0, 500, 1,500, and 3,500 ppm exposure groups, respectively, and 34 percent, 52 percent, 59 percent, and 65 percent in the females, respectively. The degree of severity tended to increase with the dose. Further information relating to protocol and additional observations are discussed in Section 5.3.3.1.1.

In the Dow Chemical Co. (1980) 2-year inhalation study in hamsters at the same levels described above for rats, a variety of gross and histopathologic observations were recorded for hamsters sacrificed at the 6-, 12-, or 18-month interim points. Histopathologically, exposure-related differences consisted of hamsters with amyloidosis of the liver, kidney, adrenals, thyroid, and spleen. Further observations are discussed in Section 5.3.3.1.3.

In the Dow Chemical (1982) 2-year inhalation study in Sprague-Dawley rats (Spartan substrain) exposed to 50, 200, and 500 ppm (174, 695, and 1,737 mg/m^3), no definite exposure-related histopathologic findings were noted. An exception occurred with females sacrificed at 15 months. Some had a focus or foci of altered liver cells. There were significant increases in non-neoplastic liver lesions (i.e., hepatocellular vacuolization and multinucleated hepatocytes) in female rats at 500 ppm (1,737 mg/m^3). Further observations are discussed in Section 5.3.3.1.4.

Histopathologic changes in liver cells of Fischer 344 rats exposed to various levels of DCM in drinking water over a 2-year exposure period have been reported by the National Coffee Association (1982). Details are described in Section 5.3.3.1.5.

5.2.2.3 Morbidity and Mortality--Heppel et al. (1944) conducted two inhalation experiments. One used 489 ppm (1,700 mg/m^3) commercial DCM for 7 hr/day, 5 days/week for 6 months. The other involved exposure for 4 hr/day, 5 days/week, for 7 to 8 weeks at a concentration of 9,789 ppm (34,000 mg/m^3). Dogs, rabbits, guinea pigs, and rats were used in each experiment. Two monkeys were added to the experiment at the higher concentration. Animals were exposed together in a single chamber. Temperature and humidity were uncontrolled. At the lower dose, 3 of 14 male guinea pigs died. They had fatty degeneration of the liver and pneumonia. No other animals' deaths attributed to DCM exposure were reported.

At the higher dose, the dog experiment was terminated after six exposures because of the continuing Stage II excitement reactions. Three rabbits and one rat died during the course of the experiment. Each animal had extensive pulmonary congestion. Clinical observations in the dogs at the lower dose showed no changes in blood pressure, blood chemistry, or liver function tests, and at autopsy, organ weights of liver, kidneys, heart, lungs, and spleen were similar to controls. No lesions due to DCM were found. All animals showed clinical effects after exposure to the higher concentration. Aside from the responses in dogs noted above, the other animals showed progressive signs of depression, usually becoming prostrate at the end of each daily exposure period. All animals, including dogs, recovered rapidly upon removal from the chamber, and fed well. At autopsy, dogs and guinea pigs showed fatty degeneration of the liver. Pulmonary congestion was found in the rabbits. Monkeys and rats showed no lesions related to DCM exposure.

5.2.2.4 Summary of Effects of Chronic Exposures on Laboratory Animals--Chronic exposure to high levels of DCM has been reported to produce alterations in behavior and in hepatic and renal function. When deaths occurred, they were frequently due to pulmonary congestion. Apparently these effects begin at about 500 to 1,000 ppm with behavioral effects in evidence early, followed by changes in internal organs. No data are available to determine if the effects seen were reversible after cessation of chronic exposures. This issue could best be addressed through additional research.

5.3 TERATOGENICITY, MUTAGENICITY, AND CARCINOGENICITY

5.3.1 Teratogenicity, Embryotoxicity, and Reproductive Effects

It is not possible, on the basis of limited available data, to define the full potential of DCM to produce adverse teratogenic or reproductive effects. Human epidemiological studies that evaluate the effects of DCM on the exposed population are difficult to conduct. Each of the available mammalian studies had methodological drawbacks that do not allow for conclusive evaluation of the ability of DCM to produce a teratogenic response over a wide range of doses, which should include doses high enough to produce signs of maternal toxicity and lower doses that do not produce this effect. Other studies in chicken embryos have indicated that DCM disrupts embryogenesis in a dose-related manner (Elovaara et al., 1979). However, since administration of DCM directly into the air space of chicken embryos is not comparable to administration of dose to animals with a placenta, interpretation of this result related to the potential of DCM to cause adverse human reproductive effects is not possible. Another preliminary study in rats indicated that adverse effects on offspring behavior may occur after maternal exposure to high levels of DCM. However, additional behavioral studies have not been conducted to evaluate this effect more fully.

5.3.1.1 Animal Studies--The following discussion subscribes to basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized by the Office of Pesticides and Toxic Substances (U.S. EPA, 1980):

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic"

has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumerary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Environmental Protection Agency (EPA) suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which is irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to *in utero* exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up." Unless a series of specific kidney function tests is performed on the neonate, however, no conclusion may be drawn concerning permanent organ functional changes. This same uncertainty as to possible long-lasting after effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

5.3.1.2 *Mice*--Swiss-Webster mice (30 to 40) were exposed via inhalation to 1,250 ppm (4,342 mg/m^3) of DCM (97.9 percent pure) for 7 hr/day during days 6 through 15 of gestation (Schwetz et al., 1975). Two control groups were similarly exposed to filtered room air. Day 0 of gestation was determined when a vaginal plug was observed. Caesarean sectioning of dams was performed on day 18 of gestation.

Dams were evaluated for body weight gain and various organ weights. Maternal body weights were recorded on days 6, 10, and 16 of gestation, as well as on the day of caesarean section. Maternal COHb level determinations were performed on blood samples collected via orbital sinus puncture immediately following the third and tenth (last) exposure. Following caesarean sectioning, fetuses were weighed, measured (crown-rump length), sexed, and examined for external malformation. One-half of the fetuses in each litter were examined for soft-tissue malformations (free-hand sectioning) and one-half were examined, following staining, for skeletal malformations. One fetus in each litter was randomly selected and evaluated using histological techniques following serial sectioning.

In this study, maternally toxic effects of DCM exposure were observed, consisting of a significant increase in body weight, a significant increase in absolute liver weight, and significant increases in COHb values with return to control levels after 24 hours. On the basis of the maternal liver weight observations, a minimal toxicity may have occurred. The cause of the increased maternal weights is unknown.

Of the 12 litters examined, a statistically significant number of litters contained fetuses that had a single extra center of ossification in the sternum. This common variation in mice is thought to reflect the degree of embryonic development. It is not known if this observation resulted from an acceleration in development or was a chance occurrence. The litters in exposed group were heavier than control fetuses (5.74 g vs. 5.42 g); however this may be caused by the average slightly smaller litter size compared to controls (10 vs. 12). The litters in this treatment group also had a lower incidence of delayed ossification of the sternebrae (17 vs. 23 percent), split sternebrae (8 vs. 18 percent), and ossification of the skull bones (25 vs. 36 percent). Cleft palate and "rotated kidney" were observed in two (17 percent) of the DCM exposed fetuses, but not in any of the control litters. Because of the low incidence of these effects, these effects may reflect spontaneous malformation rates.

5.3.1.3 Rats--A study using the same design as that used for the mice (Section 5.3.1.2) was performed in Sprague-Dawley rats (Schwetz et al., 1975). Rats inhaled DCM at 1,250 ppm (4,342 mg/m^3) for 7 hr/day on days 6 through 15 of gestation, with day 0 being the day spermatozoa were observed in vaginal smears. Dams were Caesarean sectioned on day 21 of gestation. All other procedures were identical to those performed in the mouse study with the exception that, in the rat study, food consumption was monitored.

No effect on maternal body weight or food consumption was observed. The average absolute maternal liver weight was significantly increased in comparison with the control values but there was no effect on the relative weight of the liver. Carboxyhemoglobin values in the dams increased significantly during exposure but returned to control levels within 24 hours.

In the 19 litters evaluated, there was no effect on the average number of implantation sites per litter, litter size, number of resorptions, or fetal sex ratio and body weight. The incidence of dilated renal pelvis was significantly increased, but this observation might indicate a slight but reversible delay in development similar to delays in sternal ossification. However, since this study evaluated only one dosage level, it is not possible to firmly establish the significance of this effect or its reversibility.

Hardin and Manson (1980) used Long-Evans rats to evaluate the effect of exposure to DCM (97 percent pure) via inhalation at 4,500 ppm (15,633 mg/m^3) for 6 hr/day, 7 days/week to determine whether exposures before and during gestation were more detrimental to the developing conceptus than exposures before gestation only. Minimal maternal toxicity, consisting of increased absolute and relative liver weight and elevated COHb levels, was observed. The litters of rats exposed to DCM during gestation also had significantly lower fetal body weights than controls. No other significant deleterious effect was observed. Twenty animals were used to evaluate teratogenicity. Ten were used in a separate evaluation of behavioral toxicity.

Bornschein et al. (1980) reported on the behavioral teratogenic effects in the Long-Evans rats exposed to DCM from the Hardin and Manson (1980) study. Ten rats were evaluated for general activity at 5, 10, 45, and 108 days of age, avoidance learning at approximately 4 months of age, and activity following avoidance learning at approximately 5 months of age. Fetuses delivered by Caesarean section had lower fetal body weight than those that were naturally delivered. Treatment-related effects were reported for animals in the general activity tests as early as 10 days of age (both sexes) and were still demonstrable in male rats at 150 days of age. No adverse effects were observed in growth rate, long-term food and water consumption, wheel running activity, or avoidance learning.

In the Bornschein et al. (1980) study, the number of rats per test group was small, usually one male and one female per litter. Therefore, this study should be regarded as preliminary, and additional studies are needed to fully confirm these effects. Also, the entire field of behavioral teratology is in its early stages of development (Buelke-Sam and Kimmel, 1979), and the significance of alterations in behavior to human risk assessment is not clearly defined.

5.3.2 Mutagenicity

Dichloromethane has been tested for mutagenic activity in bacteria, yeast, insects, nematodes, mammalian cells *in vitro*, and rodents. These studies are discussed below and are summarized in Tables 5-4 to 5-9.

5.3.2.1 Gene Mutations

5.3.2.1.1 Bacteria. There are 14 reports in the literature concerning the mutagenic potential of DCM in bacteria; the *Salmonella* histidine reversion assay was used in all of these studies (Simmon et al., 1977; Simmon and Kauhanen, 1978; Kanada and Uyeta, 1978; Jongen et al., 1978; McGregor, 1979; Snow et al., 1979; Green, 1980; Rapson et al., 1980; Barber et al., 1980; Nestmann et al., 1980; Green, 1981; Gocke et al., 1981; Nestmann et al., 1981; and Jongen et al., 1982). Kanada and Uyeta (1978) also tested DCM in the *B. subtilis* rec assay. DCM tested positive in all studies using *Salmonella* without or with metabolic activation in strains TA100, TA1535, or TA98 when assays were performed in sealed gas tight exposure chambers. Negative responses were reported by Rapson et al. (1980) and Nestman et al. (1980) in standard assays, but these tests are judged to be inadequate because DCM was added directly to the agar medium, and no precautions were taken to prevent excessive evaporation of the test material. The tests were carried out at 37°C, which is very close to the boiling point of DCM (39°C); thus, it is likely excessive evaporation occurred. Data were presented in many of the reports, and a clear dose-related response is apparent for each, with 10-fold or greater increases in revertants observed at the highest doses compared to negative controls. The doses employed and the responses observed are summarized in Table 5-4.

The purity of the test material was not given in any report. Because of this, most positive responses must be viewed with caution; they may be caused by substances other than DCM. For instance, formaldehyde, a metabolite of DCM, could form nonenzymatically in the aqueous solutions used for biological testing by hydrolysis of DCM (March, 1977). Because of the consistency of positive responses with several different samples, it is more likely the DCM itself is mutagenic. For instance, in their tests of DCM, Barber et al. (1980) used a redistilled sample estimated to be >99.9 percent pure, containing only traces of 1,1- and 1,2-dichloroethane, chloroform, chloromethane, and an unidentified C_5H_{10} aliphatic material (Dr. E. Barber, Eastman Kodak, personal communication). Nestmann et al. (1981) reported that their sample was "gas chromatographically pure," and Gocke et al. (1981) checked their sample for the "correct melting

TABLE 5-4. MUTAGENICITY TESTING OF DCM IN BACTERIA

Reference	Test System	Strain	Activation System	Concentration/Results		Comments
Simmon et al., 1977	*Salmonella*/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	None	(Extrapolated from figure 17) 0, 50, 100, 200, 400, and 800 µl/9 liter desiccator	(Extrapolated from Figure 17) Dose (µl) TA100 Revertants/plate 0 170 50 210 100 300 200 400 400 650 800 1350	1. Toxicity not reported. 2. Number of revertants observed for TA100 not specified numerically. 3. Data not presented for strains other than TA100. 4. Purity and source of compound not provided. 5. Positive response.
Simmon and Kauhanen, 1978	*Salmonella*/S9 vapor exposure	TA100	Aroclor-1254 induced rat liver microsome S9 mix	0 and 1 ml/9 liter desiccator for 6.5 and 8 hours	TA100 revertants #h Met. Act. Treated Control 6.5 - 688 133 + 1344 130 8 - 830 174 + 912 158	1. Toxicity not reported. 2. Purity and source of compound not provided. 3. Used as a positive control in the testing of 2-chloroethyl-chloroformate. 4. Positive response.

(continued on the following page)

TABLE 5-4. (continued)

Reference	Test System	Strain	Activation System	Dose	Results		Comments
				Dose (% in air)	TA 100		
					+S9	-S9	
Green, 1980	*Salmonella*/S9 vapor exposure	TA1535 TA100	Rat liver fractions	0	69±3	-	1. Preliminary results presented in abstract form. 2. Metabolic studies conducted in rat tissue and TA100. Similar metabolism in both systems. Radiolabel reported to bind to bacterial DNA but not to rat liver DNA. 3. Purity of DCM not reported. 4. Positive response. Author thinks this is due to close proximity of cytoplasmic enzymes and intermediates to DNA in bacteria and that negative responses would be obtained in higher organisms. Positive responses in other tests argue against this. See discussion in text.
				1.4	283±10	267±20	
				2.8	506±27	462±28	
				5.5	825±34	872±27	
				8.3	1050±88	997±88	

(continued on the following page)

TABLE 5-4. (continued)

Reference	Test System	Strain	Activation System	Concentration/Results				Comments
				Atmospheric Concentration%		Plate Concentration		
				Theoretical	Actual	µg	Revertants	
McGregor, 1979	*Salmonella*/S9 vapor exposure	TA1535	None	0	nd	nd	15	1. Purity of DCM not reported. 2. Positive response.
				0.5	0.14	245	20	
				1.0	0.33	600, 595, 530	25	
				2.0	0.67	1400	50	
				4.0	1.60	2425	75	
				10	nd	nd	80	
Nestmann et al., 1980	*Salmonella*/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-induced rat liver S9					1. Data not presented. 2. Negative response in standard test. 3. Positive response in gas tight chamber. Doubling in revertant counts for TA1535; 6-fold increase for TA100.

Reference	Test System	Strain	Activation System	Dose	Results				Comments
				(µl/Chamber)	TA100**		TA98		
					+S9	-S9	+S9	-S9	
Snow et al., 1979	*Salmonella*/S9 vapor exposure	TA98 TA100	Methylene chloride induced Syrian Golden Hamster liver S9 microsome mix	0	66	63	38	19	1. Purity of DCM not reported. 2. No information about variability of results. 3. Positive response.
				100	177	142	47	31	
				300	463	274	69	46	
				500	642	468	92	61	
				1000	972	632	39	72	

**Mean calculated from three plates/dose.

(continued on the following page)

TABLE 5-4. (continued)

Reference	Test System	Strain	Activation System	Concentration	Results				Comments
Kanada and Uyeta, 1978	Salmonella/S9 and B. subtilis rec assay testing	TA98 TA100	PCB induced rat liver microsome S9 mix	Not reported	DCM reported negative for both strains in B. subtilis and positive for both in S. typhimurium				1. Results summarized in abstract form. 2. Positive results of "Ames" testing supports reports by other authors using same system.
Jongen et al., 1978	Salmonella/S9 vapor exposure	TA98 TA100	Phenobarbital induced rat liver microsome S9 mix	(ppm x 10^3)	TA100*		TA98*		1. Testing conducted in gas tight perspex boxes. 2. Only highest dose exhibited less than 83% survival. 3. Purity of DCM not reported. 4. Positive response.
					+S-9	-S9	+S9	-S9	
				0	152±19	129±12	21±4	19±5	
				5.7	329±37	248±32	54±5	44±8	
				11.4	515±76	407±47	74±4	56±10	
				14.1	757±82	582±56	93±9	66±12	
				22.8	865±82	653±89	123±10	96±11	
				57.0	1201±191	740±94	149±42	110±42	

*Results from three experiments, five plates/dose.

Reference	Test System	PPM Vapor	µmoles/plate*	Revertants/Plate						Comments
				TA1535		TA98		TA100		
				-S9	+S9	-S9	+S9	-S9	+S9	
Barber et al., 1980	Salmonella/S9 vapor exposure	0	0	23	28	23	39	254	264	1. Tested redistilled sample of DCM >99.9% pure. 2. Revertants/nmole at highest dose for TA1535, TA98, and TA100 were 0.0006, 0.006, and 0.03, respectively. 3. Data shown for testing in gas tight chamber. 4. Negative response in standard test; positive response in gas tight chamber.
		3,600	38	40	36	259	288	752	1152	
		7,200	76	59	51	441	297	1440	960	
		9,100	96	78	78	459	322	2640	1096	
		10,900	115	64	50	741	479	3060	3240	

*DCM concentration measured by GC/MS in aqueous phase of petri dish.

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TABLE 5-4. (continued)

Reference	Test System	Strain	Activation System	Concentration/Results	Comments
Nestmann et al., 1981	*Salmonella*/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor 1254-induced rat liver S9		1. Levels of DCM in exposure chambers related directly to the mutational dose-effect curves of 3 paint removers. 2. Data shown for one paint remover only. Other 2 gave similar response. 3. Purity of DCM not reported. 4. Positive response for paint removers likely due to DCM.

Material			his+ Revertants/Plate[a]			Exposure Level (mg/1)				
	Weight (mg)					DCM			Methanol	Ethanol[d]
Type	Added	Vaporized	TA1535	TA100	TA98	Time Averaged[b]	Max 6h Calculated[c]	Measured		
Paint remover	0	--	16	144	25	---	---	---	---	---
	203	144	22	310	31	12.7	15.5	13.0	<0.5	---
	370	241	14	433	42	21.9	25.5	23.0	<0.5	---
	790	469	23	563	76	40.1	49.9	45.0	0.7	---
	1435	903	31	785	60	80.2	95.4	86.0	1.9	---
		(ml)								
Mix [90:5:5 v/v/v DCM/methanol/ethanol]	0	---	13	154	32	---	---	---	---	---
	0.1	0.1	15	268	43	12.2	12.8	11.5	<0.5	<0.9
	0.2	0.2	24	401	73	26.9	25.3	27.5	<0.5	0.7
	0.4	0.4	25	789	138	50.6	50.8	50.0	0.9	1.1
	0.8	0.8	34	1084	164	94.1	101.0	94.5	2.6	2.7
4NQO (Average values from triplicate plates in 4 experiments	0.001	---	28	878	162	---	---	---	---	---

(a). Average values from triplicate plates. (b). Determined from an area under curve for concentration against time. (c). Calculated from amount vaporized assuming only DCM vaporized, in 91 chamber. (d). Maximum measured.

(continued on the following page)

TABLE 5-4. (continued)

Reference	Test System	Strain	Activation System	Dose	Results	Comments
Green, 1981	*Salmonella*/S9 vapor exposure	TA100	Aroclor-1254 induced rat liver S9, microsomes, and cytosol	% Vapor 0 2.8 5.0 8.4	Revertants +S9 -S9 100 100 458 386 700 720 950 900	1. Bacterial and mammalian metabolism similar. 2. Cytosol and glutathione catalyze DCM to formaldehyde and CO_2. S-chloromethylglutathione is a putative intermediate. 3. DCM converted to carbon monoxide in the presence of microsomes. Formyl chloride is a putative intermediate. 4. Purity of DCM not given. 5. Positive response. See entry for Green (1980).
Gocke et al., 1981	*Salmonella*/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-1254 rat liver S9	µl/desiccator 0 125 250 500 750	Revertants +S9 -S9 30±0 40±0 54±7 85±7 68±32 110±14 105±7 195±21 203±32 295±7	1. Spontaneous revertants for TA100 too low. 2. No information presented for toxicity. 3. Purity of DCM not given. 4. Equivocal positive response.
Jongen et al., 1982	*Salmonella*/S9 vapor exposure	TA100	Aroclor-1254 rat liver S9, microsomes, and cytosol	Activation --- S9 Cytosol Microsomes	% DCM 0 0.35 0.7 1.4 150 210 350 550 150 240 410 730 150 220 420 810 150 215 380 610	1. Purity of DCM not given. 2. Positive response.

point (sic) and elementary analysis." From this information we conclude that the consistent positive responses in Salmonella can be attributed to DCM. Barber et al. (1980) conducted their tests in a chemically inert, closed incubation system and analyzed the concentrations of DCM in the vapor-phase head space and in the aqueous phase of a test plate by gas-liquid chromatography (Barber et al., 1981). Based on this information, the mutagenic responses at the highest dose (i.e., 115 μmoles/plate) for TA1535, TA98, and TA100 were 0.0006, 0.006, and 0.03 revertants per μmol, respectively, indicating DCM is a weak mutagen for Salmonella under the conditions of the test.

The results discussed above clearly show that DCM is mutagenic in Salmonella. However, questions have been raised about the applicability of these results to predicting mutagenicity in other species, especially mammals. DCM is metabolized, apparently via mutagenic intermediates, to CO and CO_2 in both rodents and humans (see Chapter 4). CO is produced by oxidative dechlorination of DCM by the microsomal P_{450} mixed function oxidase system. Formyl chloride is believed to be an intermediate in this pathway. A second cytosolic glutathione transferase system dehalogenates DCM to produce formaldehyde, which is further oxidized to CO_2. This pathway is thought to proceed via an S-chloromethyl glutathione intermediate (Ahmed and Anders, 1978; Kubic and Anders, 1975). Formyl chloride and S-chloromethyl glutathione are highly reactive alkylating agents. Salmonella also metabolizes DCM to CO_2 and CO apparently by reaction pathways similar to those occurring in mammals (Green, 1980, 1981). Because of the reactivity of formaldehyde, formyl chloride, and S-chloromethyl glutathione, and the proximity of bacterial DNA to bacterial cytoplasmic enzymes, it has been hypothesized that these chemical substances are more effective as mutagens when they are formed by bacterial metabolism than when they are formed outside the bacterial cell by rat liver fractions (Green, 1980, 1981). The basis for this hypothesis is that rat liver fractions used for metabolic activation have little effect on increasing the mutagenicity of methylene chloride in the Ames test. The implication is made that as organismic complexity is increased, there is less likelihood that DCM will cause mutations. It is argued that compartmentalization of DNA into the nucleus protects the genetic material from exposure to the mutagenic metabolites of DCM (i.e., they would react with other cellular constituents first) and thus there is little or no mutagenic risk. The positive results using eukaryotes, discussed in the following sections, argue against this hypothesis.

5.3.2.1.2 Yeast. Callen et al. (1980) studied the ability of DCM obtained from Fisher Scientific Company (purity not reported) and six other halogenated hydrocarbons to cause gene conversion, mitotic recombination, and reverse mutations in *Saccharomyces cerevisiae* (Table 5-5). Strain D7 log phase cells were incubated for 1 hour in culture medium containing 0, 104, 157, and 209 mM DCM. The percent survival for these doses were 100, 77, 42, and <0.1, respectively. Due to the toxicity of the compound, the genetic end points were not measured at the highest dose. The response for the other doses (0, 104, and 157 mM) expressed per 10^6 survivors were: gene conversion at the trp-5 locus (18, 28, and 107); mitotic recombination for ade-2 (310, 190, and 4,490); total genetic alterations for ade-2 (3,300, 3,900, and 14,000); and reverse mutations for ilv-1 (2.7, 4.4, and 5.8). A greater than twofold dose-related increase over negative controls was observed for each endpoint measured. The magnitude of recombinogenic response at the *ade* locus may have been overestimated in this study because the treatment regime used for estimating the recombinants overlaps that used for estimating the number of *trp-5*$^+$ convertants. No exogenously applied metabolic activation was used in these experiments, which indicates that yeast metabolizes DCM intracellularly to a mutagenic intermediate(s) that reaches nuclear DNA. In another genetic study employing yeast, Simmon et al. (1977) reported that DCM (source and purity not given, but stated to be the highest available purity) did not increase mitotic recombination in strain D3 of *Saccharomyces cerevisiae* when cells (1×10^8) in suspension culture were exposed for 4 hours at 30°C (Table 5-5). The doses used and the actual experimental values obtained for mitotic recombination were not reported. The discrepancies between the work by Callen et al. (1980) and Simmon et al. (1977) may be due to a number of factors including the different strains used (D3 versus D7), exposure time differences (4 hours versus 1 hour), or differences in the incubation temperature (30°C versus 37°C). Callen et al. (1980) reported that an increase in the treatment time of D7 cells with DCM from 1 hour to 4 hours significantly reduced the level of genetic activity. Other variables, such as a lower level of P_{450} enzymes in strain D3, could conceivably account for the discrepancy in the results. At this time, DCM is considered to be a positive mutagen in yeast.

5.3.2.1.3 Drosophila. Two reports are available concerning the ability of DCM to induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Table 5-6). Abrahamson and Valencia (1980) reported negative results, while

TABLE 5-5. GENE MUTATIONS AND MITOTIC RECOMBINATION IN YEAST

Reference	Test System	Strain	Dose (mM)	% survival	Response/10^6 Survivors				Comments
					trp-5 Conversion	Recombination	Total Genetic Alterations	ilv-I Revertants	
Callen et al., 1980	*Saccharomyces cerevisiae*	D7	0	100	18	310	3300	2.7	1. Positive response. 2. Active metabolites produced by this system are made intracellularly rather than by an exogenously employed activation system.
			104	77	28	190	3900	4.4	
			157	42	107	4490	14000	5.8	
			209	--	--	--	--	--	
Simmon et al., 1977	*Saccharomyces cerevisiae* suspension test	D3							1. Data not provided but reported negative for mitotic recombination. 2. Strain differences and differences in treatment conditions (i.e., time and temperature) may be the cause of differences between this study and that of Callen et al. (1980). 3. Cytochrome P450 concentration not known. Callen et al. (1980) report different yeast strains have different levels.

TABLE 5-6. GENE MUTATIONS IN MULTICELLULAR EUKARYOTES *IN VIVO*

Reference	Test System	Strain	Chemical	Route	# Chromosomes Tested	# Lethals	Corrected Lethals (%)	Comments
Abrahamson and Valencia, 1980	*Drosophila* sex-linked recessive lethal test	FM6 females, Canton S males	EMS	Fed	773	44	5.69	1. No precaution taken to design exposure chambers to prevent evaporation of the compound for feeding experiment. 2. No concurrent negative controls reported for the injection experiment. 3. Negative response at dose tested (224 mM).
			Tris	Fed	2442	35	1.43	
			Neg. Controls	Fed or Inj	94491	230	0.233	
			DCM	Fed	14682	34	0.204	
				Inj	8262	18	0.157	
Gocke et al., 1981	*Drosophila* sex-linked recessive lethal test	Basc females, Berlin K males	DCM (mM)	Lethals/Brood 1	2	3		1. Positive response for Brood 1 indicating DCM is mutagenic to sperm in *Drosophila*. 2. Higher dose used than in test by Abrahamson and Valencia (1980).
			0	19/7130 (0.27)	8/5525 (0.14)	13/3416 (0.38)		
			125	16/3632 (0.44)	2/2579 (0.08)	6/1310 (0.46)		
			620	8/1213 (0.66)	3/735 (0.41)	5/1005 (0.50)		
			Total treated	24/4845 (0.50)	5/3114 (0.16)	11/2315 (0.47)		
				$P < 0.05$				

(continued on the following page)

TABLE 5-6. (continued)

Reference	Test System	Concentration (mol/L)	Mutation Frequency (Lethal mutations/10^5 loci)	Toxicity (Survival rel. to controls)				Comments
				L2 Juveniles	L2-L3 Molt	L3-L4 Molt	L4 Adult Molt	
Samoiloff et al., 1980	*Panagrelus redivivus* sex-linked recessive lethal test	DCM						1. Equivocal positive response. 2. Not dose-related. 3. Some positive controls gave negative (e.g. EMS) or only marginally positive response (e.g. 3-methylcholanthrene). 4. Test system not validated.
		10^{-8}	6.0	1.02	0.99	0.97	0.46	
		10^{-6}	10.0	1.02	1.00	0.86	0.15	
		10^{-4}	9.8	1.00	1.00	0.88	0.17	
		Proflavine						
		10^{-8}	12.5					
		10^{-6}	10.0					
		10^{-4}	28.6					

a positive response was reported by Gocke et al. (1981). Abrahamson and Valencia (1980) conducted their sex-linked recessive lethal tests using two routes of administration: adult feeding and injection. Due to the low solubility of DCM in aqueous solutions, high concentrations of the test substance were not used in these experiments, which may account for the negative response observed. In the feeding study, male Canton S flies were placed in culture vials containing glass microfiber paper soaked with a saturated solution of 1.9% DCM in a sugar solution (224 mM DCM) for 3 days. The feeding solution was added twice daily to compensate for evaporation of the compound. At this dose, there was no evidence of toxicity. After mating, chromosomes from the 14,682 offspring of treated parents and chromosomes from the 12,450 offspring of concurrent control parents were assessed for recessive lethal mutations. No evidence of mutagenicity was observed. DCM gave a level of 0.204% lethal mutations compared to 0.215% for controls. However, because of the volatility and insolubility of DCM, the actual dosing to the animals may have been much less than expected.

In the injection study, 0.3 µl of an isotonic solution containing 0.2% DCM was administered to male flies. This exposure level resulted in 30% post-injection mortality. However, the post-injection mortality observed for the controls was not reported. Because the mortality observed in studies such as this is due not only to the test chemical administered, but also to the damage caused by injection, concurrent negative controls upon which to base conclusions concerning toxicity of the test chemical are necessary. After mating, 8,262 chromosomes from the offspring of treated parents and 8,723 chromosomes from the offspring of control parents were assessed for recessive lethal mutations. No evidence of mutagenicity was observed by this route of administration. Flies injected with 0.2% DCM had 0.157% lethals compared to 0.206% for controls.

Gocke et al. (1981) also tested DCM (Merck, Darmstadt, FRG, purity not given) for its ability to induce sex-linked recessive lethal mutations in Drosophila. Two solutions, 125 mM and 625 mM in 2% DMSO and 5% saccharose, were fed to wild-type Berlin K male flies for an unreported period of time. The higher dose (625 mM) is reportedly close to the LD_{50}. These males were then mated to Basc females. Three broods were scored (i.e., offspring from virgin females mated to treated males on days 1 through 3, 4 through 6, and 7 through 10 after exposure). There were significantly more lethals (24 out of

4,845 chromosomes scored) for brood 1 (0.50% lethal mutations) compared to the negative controls (19 lethals out of 7,130 chromosomes, 0.27% lethal mutations), $P \leq 0.05$. Elevated, but not statistically significant increases in lethal mutations were noted in broods 2 (0.16 percent compared to 0.14% lethals) and 3 (0.47 percent compared to 0.39% lethals) of the treated flies compared to the controls. As noted in Table 5-6 the incidence of lethals is dose-related. The incidence of lethals for the high dose in brood 2 is elevated nearly threefold over the corresponding negative control value (0.41 versus 0.14 lethals, respectively), but this may not be a significant increase because of the small sample size (735 vials). This test indicates DCM is mutagenic to sperm in Drosophila (a multicellular eukaryote). The discrepancies in results between Abrahamson and Valencia (1980) and Gocke et al. (1981) may be due to stock-specific differences (Canton S. versus Berlin K) in the metabolic activation of DCM or more likely to the larger doses of DCM employed by Gocke et al. (1981).

5.3.2.1.4 Nematodes. In another sex-linked recessive lethal test, Samoiloff et al. (1980) tested DCM for its ability to mutate the nematode Panagrellus redivivus (Table 5-6). Individual females homozygous for the X-linked mutation b7 (coiled phenotype in liquid medium) were grown for 120 hours in the presence of several concentrations of DCM ranging from 10^{-3}M to 10^{-8}M. They were then washed and mated to S-15 males who carry an X-chromosome crossover suppressor extending at least 15 recombination units to either side of b7. One hundred female progeny were collected and mated to wild-type (C-15) males and their progeny scored for the presence of the b7 phenotype. The absence of b7 male progeny indicates lethality of the X-chromosome marked with b7 derived from a female grown on DCM. Three replicate experiments were performed. A non-dose-related increase in the level of lethals was observed in the progeny of DCM-treated worms compared to the negative controls. For worms treated with 10^{-8}, 10^{-6}, and 10^{-4}M DCM, the corresponding lethal mutations/10^5 loci were 6.0, 10.1, and 9.8, respectively, compared to an estimated spontaneous mutation frequency of 2.2×10^{-6} mutations/locus. Some of the positive controls tested concurrently, such as proflavine, yielded a positive response (12.5, 10.0, and 28.6 lethals/10^5 loci at 10^{-8}, 10^{-6}, and 10^{-4}M, respectively). But others, such as aflatoxin B and ethyl methanesulfonate (EMS), did not cause an increase in lethal mutations. The investigators suggest that DCM is mutagenic in nematodes, but firm conclusions cannot be made because the assay is not validated and, more importantly, because of the negative responses obtained with some of the positive controls.

5.3.2.1.5 Mammalian cells in culture. Jongen et al. (1981) tested DCM for its mutagenic potential in several mammalian cell culture tests. Testing for the induction of forward mutations at the HGPRT locus is described here (Table 5-7). Testing for the ability of DCM to cause sister chromatid exchanges (SCE), unscheduled DNA synthesis (UDS), and inhibition of DNA synthesis (IDS) will be discussed later in the section on other indicators of DNA damage.

In their testing of the ability of DCM to cause forward mutations, Jongen et al. (1981) incubated log phase CHO and V79 cells with 1, 2, 3, 4, and 5% DCM or 1, 2, 3, and 4% DCM, respectively, at 37°C for 1 hour in a closed glass container without exogenous S9 mix. DCM was obtained from Merck (analytical grade). The cells were exposed to gaseous DCM and then DCM in solution for 15-minute intervals each by alternately tilting the plates then placing them horizontally. After growth to allow for an 8-day (CHO cells) or 6-day (V79 cells) expression period, mutant cells were selected in thioguaninecontaining medium. DCM failed to increase the mutation frequency of either cell line at any dose compared to controls. However, DCM was not very cytotoxic to either cell line. At the highest dose, survival decreased only 20 percent. It would be appropriate to repeat the experiment using higher doses of DCM. EMS yielded a positive, dose-dependent increase in mutation induction in V79 cells, but it was not tested in CHO cells.

Based on the positive responses in bacteria, yeast, and *Drosophila*, and the suggestive positive response in the nematode, DCM is judged to be capable of causing gene mutations. Metabolic activation to highly reactive mutagenic metabolites apparently accounts for this response, and although these are thought to be unstable, they seem to be capable of interacting with genetic material of both prokaryotes and eukaryotes.

5.3.2.2 Chromosomal Aberrations--Three studies on the ability of DCM to cause chromosomal aberrations were evaluated. Burek et al. (1984) subjected 4 groups of 10 Sprague-Dawley albino rats (Spartan substrain, SPF-derived, 5 males and 5 females) to 0, 500, 1,500, or 3,500 ppm (0, 1735, 5205, 12,145 mg/m^3) DCM by inhalation 6 hr/day, 5 days/week for 6 months. The animals were then sacrificed, bone marrow cells were collected, chromosome preparations were made, and slides were coded and analyzed. Two hundred metaphases per animal were scored and aberrations were tabulated (See Table 5-8). No increase

TABLE 5-7. GENE MUTATIONS IN MAMMALIAN CELLS IN CULTURE

Reference	Test System	Concentration (%)	V79 Mutants/10^5 Survivors	V79 Survival (%)	CHO Mutants/10^5 Survivors	CHO Survival (%)	Comments
Jongen et al., 1981	6-Thioguanine resistance in V79 and CHO cells	DCM 0	2	100	1.9	100	1. Equivocal negative response. 2. Highest dose only resulted in 20% decrease in survival. Higher doses up to about 80% toxicity should be tested.
		1	1.8	98	1.8	90	
		2	2	95	1.2	85	
		3	1.7	85	0.9	80	
		4	1.6	80	2.1	73	
		5			2.5	76	
		EMS 0	2				
		2	13				
		4	33				

in the total frequency of abnormal cells or in the frequency of any specific type of aberration was noted in the treated animals compared to the controls. There were 1.1 ± 1.3, 0.6 ± 0.7, 0.8 ± 1.2, and 1.1 ± 0.9% cells with aberrations in animals treated with 0, 500, 1,500, and 3,500 ppm (0, 1735, 5205, 12,145 mg/m^3) DCM, respectively.

Thilagar and Kumaroo (1983) treated CHO cells grown in either plastic or glass culture flasks with 0, 2, 5, 10 and in one experiment 15 µl/ml (i.e., 0, 31, 78, 156, and 234 mM) DCM for 2 hours with or 12 hours without S9 mix derived from Aroclor-induced rat livers. DCM was obtained from Fisher Scientific (certified A.C.S., lot no. 713580). After the exposure period, the cells were washed, refed, and allowed to grow before being arrested at metaphase with colcemid and harvested for chromosome preparation. Slides were coded and read "blind;" 100 cells were scored for each dose level (50 cells/duplicate flask). DCM induced a dose-related increase in chromosome aberrations (see Table 5-8) ranging from 0.02 aberration/cell in the negative controls to 1.44 aberrations/ cell at 15 µl/ml(234 mM). The response was not dependent on the presence of the exogenous metabolic activation system.

Gocke et al. (1981) assessed the ability of DCM (Merck, Darmstadt; purity not given) to cause micronuclei in polychromatic erythrocytes (PCE). Two male and two female NMRI mice were used for each of three dose levels (425, 850, and 1,700 mg/kg per intraperitoneal injection). The highest dose approximated the LD_{50} for mice. Intraperitoneal injections of each dose were given at 0 and 24 hours, the animals were sacrificed at 30 hours, bone marrow smears were made, and 1,000 PCEs per animal were scored for the presence of micronuclei. An increase in PCEs with micronuclei was observed at the two highest doses, but the response was not dose-related and was not double the control value. Thus, the results are considered suggestive of a positive response but are not conclusive. (There were 0.19% micronuclei in the untreated controls compared to 0.35% micronuclei in the animals receiving two injections of 850 mg/kg, and 0.28% micronuclei at the highest dose).

Based on the positive response reported by Thilagar and Kumaroo (1983), DCM is tentatively judged to be capable of causing chromosomal aberrations. The *in vivo* negative responses reported by Dow Chemical Company (1980) and Gocke et al. (1981) are not readily comparable with the *in vitro* results.

5.3.2.3 Other Indicators of DNA Damage

5.3.2.3.1 Sister chromatid exchange (SCE). Two papers have been published on the ability of DCM to induce SCEs (Table 5-9). Jongen et al. (1981) tested

TABLE 5-8. TESTS FOR CHROMOSOMAL ABERRATIONS

Reference	Strain/Tissue	Route of Exposure	Dose	Breaks Chromatid	 Chromosome	Dicentrics	Rings	Exchanges	Comments
Dow Chemical Company, 1980	Male and female Sprague-Dawley rat/bone marrow	Inhalation	0	0.9 ± 0.99	0.2 ± 0.42	0	0	0	1. 5 animals/sex/dose. 2. 200 cells/animals. 3. Dose to bone marrow cells may have been low. 4. Negative response.
			500	0.5 ± 0.71	0.2 ± 0.42	0	0	0	
			1500	0.5 ± 0.97	0.1 ± 0.32	0	0	0.1 ± 0.32	
			3500	0.7 ± 0.48	0.2 ± 0.42	0	0.2 ± 0.42	0	

Reference	Test System	Dose	RCG*	Breaks Chromatid	 Isochromatid	Exchange	Number of Aberrations/Cell	Cellsw/ Aberration (%)	Comments
Thilagan and Kumaroo, 1983	Cultured CHO cells	DCM (µl/ml)							1. Positive response. 2. Four experiments yielded similar response.
		0	100	2	0	0	0.02	2	
		2	98.4	4	0	2	0.06	6	
		5	75.3	8	14	8	0.34	26	
		10	66.7	12	34	10	0.56	38	
		TEM 1 µg	N.D.	22	30	42	0.96	66	

*RCG = Relative cell growth.

Reference	Strain/Tissue	Route of Exposure	Dose ppm	Breaks Chromatid	 Chromosome	Dicentrics	Rings	Exchanges	Comments
Gocke et al., 1981	Male and female NMRI mice/bone marrow	i.p. Injection	No. injection x mg/kg		Micronucleated Polychromatic Erythrocytes (%)				1. Suggestive positive response; authors indicate negative response. 2. Dose to bone marrow cells may have been low.
			0			0.19			
			2 x 425			0.19			
			2 x 850			0.35			
			2 x 1700			0.28			

the ability of 0.5, 1.0, 2.0, 3.0, and 4% DCM (i.e., 58, 118, 235, 353, and 471 mM) to induce SCEs in V79 cells. Log phase cells were incubated at 37°C for 1 hour in a closed glass container. The cells were exposed to DCM in the gaseous phase and in the medium by tilting the plates for 15 minutes, then placing them horizontally. The experiment was conducted seven times and each yielded a dose-related increase in SCEs/cell, which approached but did not exceed a twofold increase above the control level. An analysis of variance of effects of different doses within experiments showed the increases in frequency of SCEs to be statistically significant ($p < 0.001$). Increasing the exposure time to 2 hours or 4 hours or using S9 from rat liver did not alter the shape of the dose-response curve, which plateaued at 1% DCM. The authors suggest that this phenomenon is due to a saturation of the metabolic activation system of V79 cells.

Thilagar and Kumaroo (1983) exposed CHO cells to 0, 2, 5, 10, and in one experiment 15 ul DCM/ml of medium (0, 31, 78, 156, and 234 mM DCM) for 2 hours with and 24 hours without metabolic activation. The cells were grown for 24 hours in BrdUrd followed by a mitotic shake off, fixing, and staining by a fluorescence-plus-Giemsa technique, and then the coded slides were scored "blind." Slight dose-related elevations in SCE values were noted (see Table 5-9), but they never exceeded a 50% increase at the highest dose. The authors judged their test to be negative, but the concentrations were lower than used by Jongen et al. (1981).

McCarroll et al. (1983) reported in an abstract that consistent and dose-related increases were observed in SCEs in CHO cells following 24-hour exposures to 1, 3.6, 5.4, and 7.0% atmospheres of DCM. A 7% atmosphere was required to elicit a statistically significant increase. Based on the reports of Jongen et al. (1981), Thilagar and Kumaroo (1983), and McCarroll et al. (1983), DCM is capable of causing DNA damage that results in SCEs.

5.3.2.3.2 DNA repair assays. In their study of the genotoxic potential of DCM, Jongen et al. (1981) also measured UDS and IDS in V79 cells and primary human fibroblasts (AH cells). These experiments were conducted by exposing 10^5 cells attached to glass coverslips (UDS assay) or to glass petri plates (IDS assay) to 0.5, 1.0, 2.0, 3.0, and 5.0% DCM (58, 118, 235, 353, and 471 mM, respectively) without metabolic activation. UDS experiments were done in duplicate, and at least 25 nuclei of non-S phase cells were scored for the number of silver grains/nucleus at each dose level. DCM had no detectable effect on UDS in either cell line. In the IDS assays, the relative rate of

TABLE 5-9. TESTS FOR SISTER-CHROMATID EXCHANGE

Reference	Test System	Dose	Results		Comments
			SCE/Cell Experiment #		
		% DCM	1	7	
Jongen et al., 1981	SCE/V79 cells in culture	0	0.26 ± 0.02	0.30 ± 0.03	1. Exposure time 1 hour. 2. Positive response. Significant increases in SCEs ($P < 0.001$). 3. Same type of dose-response observed in experiments 2-6 (data not shown). 4. DMSO did not increase incidence of SCE.
		0.5	0.40 ± 0.02	---	
		1.0	0.46 ± 0.02	0.47 ± 0.02	
		2.0	0.45 ± 0.03	0.51 ± 0.03	
		3.0	---	0.58 ± 0.03	
		4.0	0.51 ± 0.03	0.61 ± 0.03	

Reference	Test System	Dose	SCE/Cell ($\bar{X}$ ± SD)	Range of SCE's	M_1	M_1 +	M_2	Comments
Thilagar and Kumaroo, 1983	Cultured CHO cells	DCM (µl/ml)						
		0	10.28 ± 3.17	5-17	0	3	97	1. Marginal, but not significant increases in SCEs.
		2	11.36 ± 3.09	3-19	0	16	84	2. Three other experiments yielded similar responses.
		5	12.56 ± 2.95	7-18	6	54	40	3. Results not inconsistent with test by Jongen et al. (1981) where highest dose was three times greater (i.e., 471 mM vs. 156 mM).
		10	12.36 ± 3.35	7-21	4	56	40	
		Triethylene melamine 0.025 µg/ml	47.74 ± 4.76	39-61	4	49	47	

DNA synthesis was determined radioisotopically immediately after DCM exposure and 0.5, 1.5, and 3.5 hours later. The average of duplicate samples revealed that DCM inhibited DNA synthesis in V79 and AH cells at all dose levels compared to controls but that synthesis recovered with time after exposure in all cases. This is unlike the persistent inhibition of DNA synthesis by the positive control 4-nitroquinoline-1-oxide. The authors conclude that DCM was not inducing genetic damage in cells but was inhibiting DNA synthesis by an effect on cell metabolism.

Perocco and Prodi (1981) also performed a UDS assay using DCM. They collected blood samples from healthy individuals for their studies, separated the lymphocytes, and cultured 5 x 10^5 cells in 0.2-ml medium for 4 hours at 37°C in the presence or absence of DCM (Carlo Erban, Milan, Italy or MerckSchuchardt, Darmstadt, FRG, 97 to 99 percent pure). The tests were conducted both in the presence and in the absence of PCB-induced rat liver S9 mix. A comparison was made between treated and untreated cells for scheduled DNA synthesis (i.e., DNA replication) and UDS. No difference was noted between the groups with respect to scheduled DNA synthesis measured as dpm of [^{3}H] deoxythymidylic acid (TdR) after 4 hours of culture (2,661 ± 57 dpm in untreated cells compared to 2,356 ± 111 dpm in cells treated with 5 µl/ml [78 mM] DCM). Subsequently, 2.5, 5, and 10 µl/ml (39, 78, and 156 mM) DCM was added to cells cultured in 10 mM hydroxyurea to suppress scheduled DNA synthesis. The amount of unscheduled DNA synthesis was estimated by measuring dpm from incorporated [^{3}H]TdR 4 hr later. At 10 µl/ml DCM, 532 ± 31 and 537 ± 39 dpm were counted without and with exogenous metabolic activation, respectively. Both values were lower than corresponding negative controls of 715 ± 24 and 612 ± 26 dpm, respectively. No positive controls were run to ensure that the system was working properly, although testing of chloromethyl methyl ether (CMME) with activation resulted in a doubling of dpms over the corresponding negative control values (1,320 ± 57 at 5 µl/ml CMME _versus_ 612 ± 26 untreated). The authors calculated an effective DNA repair value (r) for each chemical based on the control and experimental values with and without metabolic activation. DCM was evaluated by the authors as negative in the test, but they did not state their criteria for classifying a chemical as positive. None of the experimental values from cells treated with DCM had higher dpm values than the controls.

Based on these experiments there is no evidence that DCM specifically inhibits DNA synthesis or causes UDS.

5.3.2.4 Summary and Conclusions. Dichloromethane has been tested for its ability to cause gene mutations (in Salmonella, yeast, Drosophila, Panagrellus, and cultured mammalian cells), chromosomal aberrations (in rats, mice, and cultured mammalian cells), and other indicators of DNA damage in cultured cells (sister chromatid exchange, unscheduled DNA synthesis, and inhibition of DNA synthesis).

Commercially available samples of DCM gave positive results in Salmonella, yeast, and Drosophila. The responses were weak under treatment conditions used and were obtained without the addition of metabolic activation systems (e.g., S9 mix). The data suggest that DCM may be metabolized in vivo to a mutagenic metabolite(s). Some negative results have been reported for gene mutation tests in fungi (Saccharomyces) and mammalian cells in culture, but these may represent false negative results because of the treatment conditions used. DCM has also been reported to induce chromosomal aberrations in cultured mammalian cells but not in bone marrow cells from animals exposed in vivo, perhaps because a sufficient dose of DCM did not reach the bone marrow. DCM causes a weak increase in SCEs but has not been shown to cause UDS or inhibit DNA synthesis.

Mutagenicity tests of DCM have given positive responses in four different organisms based on the weight of available evidence. DCM is judged to be a mutagen with the potential of inducing gene mutations in exposed human cells. A positive response in cultured mammalian cells indicates that DCM also causes chromosomal aberrations, but additional testing in another in vivo or in vitro chromosomal aberration assay is needed to confirm the available data. If such tests are conducted, care should be taken to ensure that the test cells are exposed to sufficiently high doses of DCM, otherwise false negative responses may be obtained.

5.3.3 Evaluation of the Carcinogenicity of DCM

The purpose of this section is to provide an evaluation of the likelihood that DCM is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact, including a potency evaluation in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior, are important to the qualitative and quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. This section presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and finally, a summary and conclusions section dealing with all of the relevant aspects of the carcinogenicity of DCM. Further, the National Toxicology Program (NTP) rat and mouse gavage bioassay draft technical report (1982) on DCM was cancelled because of data discrepancies at their contract laboratory (memo from John A. Moore dated July 25, 1983).

5.3.3.1 Animal Studies

5.3.3.1.1 Dow Chemical Company (1980) inhalation study in rats. A total of 1,032 male and female Sprague-Dawley rats (129/sex for each exposure concentration) were exposed by inhalation to DCM at 0, 500, 1,500, or 3,500 ppm (0, 1,735, 5,205, or 12,145 mg/m^3) for 6 hr/day, 5 days/week (excluding holidays), in a 2-year toxicity and oncogenicity study. Approximately 95 rats of each sex for each exposure concentration were part of the chronic toxicity and oncogenicity portion of the study. This number also included those animals that died spontaneously, were killed moribund during the study, or were killed at the end of the 2-year exposure. The remaining animals were sacrificed as part of the cytogenetic studies or for one of the interim kills at either 6, 12, 15, or 18 mo of exposure. The rats were received at 6 to 7 weeks of age (males weighed 220 to 250 g; females weighed 170 to 200 g) from Spartan Research Animals, Inc., Haslett, Michigan, and were individually marked for identification with metal ear tags. All rats were maintained on a 12-hour light/dark cycle. They were observed daily, including weekends and holidays, for general health status and signs of possible toxicity.

Dichloromethane representative of technical grade material was obtained from Dow Chemical Company, Plaquemine, Louisiana, and was used throughout the exposure. Fourteen different samples of DCM were analyzed during the 2 years of animal exposure; each sample showed 99% pure DCM, with a few trace chemical contaminants that varied slightly from sample to sample, as shown in Table 5-10. The concentration of DCM vapor in chambers was considered well within the range of expected variability. Hematologic determinations, serum clinical chemistry, urinalysis, bone marrow collection, and blood carboxyhemoglobin (COHb) determination were done in animals sacrificed at 6, 12, 15, and 18 mo (interim kills). Plasma estradiol determination was done at the 12- and 18-mo interim kills. This included samples from six controls/sex and four high-exposure animals (3,500 ppm)/sex from the 12-mo kill, which were pooled

TABLE 5-10. ANALYTICAL ANALYSIS OF DICHLOROMETHANE

	Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Specific gravity	1.320	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCl, ppm	14.2	1.8	2.3	2.9	1.8	1.1	2.5	--	--	1	--	1	1	1
H_2O, ppm	207	560	37	55	52	27	112	--	--	324*	--	264*	340*	3
Nonvolatile material, ppm	ND	<7	<7	<7	<7	<7	<7	ND	ND	ND	ND	ND	ND	ND
Methyl chloride, ppm	<1	<4	<4	4.5	4.5	4.5	4.5	1	1	1	1	1	1	1
Chloroform, ppm	60	<27	399	48	48	48	32	52	576	64	562	515	547	460
Vinylidene chloride, ppm	60	52	53	90	75	65	60	66	72	62	78	70	77	72
Trans 1,2-dichloroethylene, ppm	560	561	266	706	550	550	487	--	321	653	323	318	337	298
Cyclohexane, ppm	365	385	247	467	365	374	335	399	266	426	262	268	288	242
Ethyl chloride, ppm	6	9	5	11	8	11	5	2	2	2	2	6	1.5	6
Vinyl chloride, ppm	<1	--	--	--	--	--	--	1	1	1	1	1	1	1
Methyl bromide, ppm	23	--	--	--	--	--	--	1	1	1	1	1	1	1
Carbon tetrachloride, ppm	<2	--	--	--	--	--	--	1	15	1	13	16	20	12

*Original analysis was lost; sample was subsequently reanalyzed.

ND = Not determined.

-- = not detected.

together (two animals/sample) to give three control samples and two high exposure (3,500 ppm) samples/sex. Ten individual samples/sex (not pooled) from the high exposure and control groups were also sent from the 18-mo kill.

All animals that either died spontaneously, were killed in moribund condition, or were killed at the interim or terminal kills were subjected to complete gross and microscopic pathological examinations by a veterinary pathologist. Liver samples for possible electron microscopic evaluation were collected.

In females exposed to 3,500 ppm, there was a statistically significant increase of mortality from the 18th through the 24th mo that may be exposure related. The remaining treated groups in males or females did not differ significantly from the controls (Table 5-11). There was no exposure-related difference in body weights of either male or female rats exposed to 500, 1,500, and 3,500 ppm DCM.

Although some hematologic values were increased and others were decreased, the mean values were within the normal range of biological variability. Serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), and serum alkaline phosphatase (AP) values were in the normal range. It is noted that the females had significantly increased ($P < 0.025$) plasma estradiol levels at 18 mo, which may be related to the higher incidence of mammary tumors in the exposed (3,500 ppm) group. Urinalysis findings were in the normal range, with the exception of a few statistically significant values in specific gravity in males exposed to 1,500 ppm at 6 mo and males and females exposed to 3,500 ppm at 12 mo. Rats exposed to 500, 1,500, or 3,500 ppm had elevated COHb values but with no evidence of either dose-response or increased values with prolonged exposure.

5.3.3.1.1.1 Gross and histopathologic observations of rats from the 6-, 12-, 15-, and 18-month interim kills. Numerous gross and histopathologic observations were recorded for control and DCM-exposed rats at each time period, and most were typical of spontaneous or naturally-occurring lesions normally seen in rats of this strain. There were many palpable masses in males and females. Some palpable masses appeared to be abscesses of the preputial or clitoral glands, while others were cyst-like lesions of the skin. The total number of masses in the 3,500 ppm (12,145 mg/m^3) group of males was significantly increased over the controls at 15, 18, and 21 mo, but not at

TABLE 5-11. CUMULATIVE PERCENT MORTALITY OF RATS 2-YEAR DICHLOROMETHANE INHALATION STUDY

Month of study	DCM concentration, ppm							
	Males				Females			
	0	500	1,500	3,500	0	500	1,500	3,500
1	0.8	0	0	0	0	0	0	0
2	0.8	0	0	0.8	0	0	0	0
3	0.8	0	0	2.3	0.8	0	0	0
4	0.8	0.8	0	3.9	0.8	0.8	0.8	0
5	0.8	0.8	0	4.7	0.8	0.8	0.8	0.8
6	0.8	1.6	0.8	4.7	0.8	0.8	0.8	0.8
7	0.9	2.6	0.9	6.0	0.9	1.7	1.7	0.9
8	0.9	3.4	0.9	6.0	0.9	2.6	2.6	0.9
9	1.7	3.4	0.9	6.0	3.4	3.4	1.7	2.6
10	1.7	4.3	1.7	6.0	5.1	5.1	2.6	2.6
11	1.7	6.0	3.4	6.8	5.1	6.0	4.3	4.3
12	2.6	6.8	4.3	8.5	6.0	6.8	5.1	6.0
13	2.7	10.9	5.5	9.8	7.2	8.2	8.2	12.5
14	3.6	10.9	10.0	15.2*	9.0	8.2	8.2	12.5
15	10.9	11.8	13.6	21.4	13.5	10.9	10.0	19.6
16	15.2	16.2	20.0	29.0*	14.2	15.2	13.3	20.6
17	23.8	20.0	31.4	33.6	23.6	20.0	20.0	29.9
18	32.4	36.2	37.1	43.0	26.4	29.5	29.5	42.1*
19	44.2	45.3	49.5	54.6	36.5	42.1	46.3	57.7*
20	48.4	52.6	63.2	58.8	42.7	50.5	55.8	68.0*
21	56.8	56.8	74.7*	72.2*	49.0	58.9	65.3*	81.4*
22	63.2	65.3	83.2*	80.4*	62.5	64.2	73.7	86.6*
23	80.0	73.7	89.5	87.6	70.8	65.3	81.1	90.7*
24	85.3	85.3	93.7	92.8	78.1	74.7	86.3	95.9*

*Significantly different from control by Fisher's Exact Test, $p < 0.05$.

Source: Dow Chemical Company, 1980.

23 months. Female rats exposed to 500, 1,500, and 3,500 ppm showed an exposure-related increase in total number of masses. There was also a trend of increased benign mammary tumors in females exposed to 1,500 and 3,500 ppm. The total numbers of animals with benign mammary gland tumors were 9/28 (0 ppm), 10/29 (500 ppm), 11/29 (1,500 ppm), and 14/27 (3,500 ppm), whereas the total numbers of benign mammary gland tumors were 17/28 (0 ppm), 17/29 (500 ppm), 28/29 (1,500 ppm, $p = 9.23 \times 10^{-4}$), and 37/27 (3,500 ppm, $p = 2.33 \times 10^{-10}$). These observations were apparent only when the cumulative results of the 6-, 12-, 15-, and 18-mo kills were evaluated.

A few other observations appeared to reflect exposure-related lesions. The liver was the only organ that exhibited definite exposure-related non-neoplastic effects in both males and females at all exposure concentrations. Grossly, the effect was most prominent in females exposed to 3,500 ppm and consisted of increased numbers of dark or pale foci. The control group had an incidence of 0/28, while the 3,500 ppm DCM-exposed female rats had a significantly greater number of foci (11/27). Some rats from the 3,500 ppm exposure group had mottled livers or had an accentuated lobular pattern to the liver (0/29 control males compared to 6/27 exposed males). Because of the limited number of rats at each interim kill, this latter change may be related to exposure but may also be due to biological variability.

Histologically observed, exposure-related lesions were present in the livers of both males and females exposed to 500, 1,500, or 3,500 ppm. In males, the total number of animals with any degree of vacuolization consistent with fatty changes were 5/29 (0 ppm), 19/29 (500 ppm, $p = 2.05 \times 10^{-4}$), 21/29 (1,500 ppm, $p = 2.47 \times 10^{-5}$), and 23/27 (3,500 ppm, $p = 2.81 \times 10^{-7}$). The livers of female rats also had alterations considered to be related to DCM exposure. The total numbers of females with any degree of vacuolization consistent with fatty changes were 13/28 (0 ppm), 20/29 (500 ppm, $p = 7.16 \times 10^{-2}$), 20/29 (1,500 ppm, $p = 7.16 \times 10^{-2}$), and 22/27 (3,500 ppm, $p = 7.16 \times 10^{-3}$). Because of these effects, it may be considered that this experiment was performed at the maximum tolerated dose (MTD).

5.3.3.1.1.2 Gross and histopathologic observations of rats killed moribund or dying spontaneously during the study and those from terminal sacrifice (24 months).

Non-neoplastic observations--The liver was affected in both males and females exposed to 500, 1,500, or 3,500 ppm. The percentage of total rats with any degree of vacuolization was 17, 38, 45, and 54% in the males of the 0, 500, 1,500 and 3,500 ppm exposure groups, respectively, and 34, 52, 59, and 65% in the females, respectively. Also, the degree of severity tended to increase with the dose. The males in all exposure groups had fewer cases of grossly observed mottled and/or enlarged adrenals. These gross alterations appeared to correspond to the histologically observed decrease in the number of cases of adrenal cortical necrosis, but the nodular hyperplasia incidence (unilateral or bilateral) was increased: 18/95 (0 ppm), 30/95 (500 ppm, $p = 3.07 \times 10^{-2}$), 31/95 (1,500 ppm, $p = 2.2 \times 10^{-2}$), and 24/97 (3,500 ppm).

Tumor or tumor-like lesions--The Sprague-Dawley strain of rats used in this study historically has had a high spontaneous incidence of benign mammary tumors. The incidence varies slightly from study to study, but normally exceeds 80% in females and about 10% in males by the end of a 2-yr study. The mammary gland tumors have been classified, based on their predominant morphological cellular pattern, as fibromas, fibroadenomas, or adenomas.

The benign mammary tumor response was present in males and, to a lesser extent, in females. There was a non-statistically significant increase in the number of male rats with a benign mammary tumor exposed to 3,500 ppm (14/95 as compared to 7/95, 3/95, and 7/95 in the 0, 500, or 1,500 exposure groups). There was a slight increase in the total number of benign mammary tumors in males exposed to 0 ppm (8/95), 500 ppm (6/95), 1,500 ppm (11/95), or 3,500 ppm (17/97, $P = 4.6 \times 10^{-2}$).

The total number of female rats with a benign mammary tumor did not increase in any exposure group (0, 500, 1,500, or 3,500 ppm groups had totals of 79/96, 81/95, 80/95, and 83/97 benign mammary tumors, respectively). However, the total number of benign mammary tumors increased in an exposure-related manner, with 165/96 in the controls, and 218/95, 245/95, and 287/97 in the females exposed to 500, 1,500, and 3,500 ppm, respectively. Expressed another way, the average number of benign mammary tumors per tumor-bearing female rat increased from 1.7 in the control rats, to 2.3 in rats exposed to 500 ppm, to 2.6 in those exposed to 1,500 ppm, and to 3.0 in those exposed to 3,500 ppm. This effect is exposure related, and a dose-response relationship was apparent. There was no indication of an increased number or incidence of malignant mammary tumors in either males or females.

The number of malignant tumors (Table 5-12) increased in male rats exposed to 3,500 ppm. This increase did not appear to correlate clearly with an increased number of any one tumor type or location. However, this observation led Dow Chemical Co. (1980) to re-evaluate the gross and histopathologic data on all tumors arising in or around the salivary glands. Table 5-13 lists the specific individual animal data for these salivary gland area tumors, showing the palpable mass data, specific histopathologic diagnoses, and the number of sarcomas with metastases. Table 5-14 summarizes the incidence of salivary gland region sarcomas in male rats.

Grossly, these tumors were large (several centimeters in diameter), cystic, necrotic, or hemorrhagic. They appeared to invade all adjacent tissues in the neck region, and often completely replaced the normal salivary gland tissue. Histologically, all were sarcomas. They were composed of cells that varied from round to spindle-shaped, but that appeared to be of mesenchymal cell origin. Mitotic figures were frequently observed, as were necrosis and local invasion into adjacent tissues. Most tumors had remnants of normal salivary acini or ducts that were caught up in the cellular proliferation. Two were relatively small masses, and appeared to be arising in the interstitial and capsular tissue of the salivary glands.

One tumor of this type was found in the controls (1/93) compared to 0/94 in the 500 ppm exposure group, 5/91 in males from the 1,500 ppm exposure group, and 11/88 in males from the 3,500 ppm exposure group ($P = 0.002$). Historically, a spontaneous incidence (0 to 2%) of this tumor type has been observed in Dow's laboratory. Therefore, the 12.5% incidence (11 of the 88 rats, Table 5-14) found in the males from the 3,500 ppm group was higher than the corresponding controls of this study, and was higher than expected based on historical control data for male rats of this strain. Also, the males exposed to 1,500 ppm had five of these tumors, which was also slightly higher than expected, but was not statistically significant. Therefore, this effort appeared to be exposure related in the males exposed to 3,500 ppm.

The total number of male rats with malignant tumors was similar in the control, 500 ppm, and 1,500 ppm exposure groups. Males exposed to 3,500 ppm had an increase in this category, since 69 of the 124 rats had malignant tumors compared to 57, 59, and 57 in the 0, 500, and 1,500 ppm exposure groups, respectively.

In 1984, Burek et al. published the results of the Dow Chemical Company (1980) study.

TABLE 5-12. SUMMARY OF TOTAL TUMOR DATA FOR RATS ADMINISTERED DICHLOROMETHANE FOR 2 YEARS BY INHALATION

		Males				Females		
	Concentration in ppm	Interim kill	Spontaneous deaths and killed moribund	Terminal kill	Cumulative totals	Spontaneous deaths and killed moribund	Terminal kill	Cumulative totals
Total number of rats examined	0	29	81	14	124	75	21	124
	500	29	81	14	124	71	24	124
	1500	29	89	6	124	82	13	124
	3500	27	90	7	124	93	4	124
Total number of rats with a tumor	0	9	53	13	75	67	21	102
	500	7	52	13	72	68	24	107
	1500	9	67	6	82	79	13	106
	3500	11	68	7	86	91	4	109
Total number of rats with a benign tumor	0	6	33	11	50	65	21	100
	500	3	29	11	43	60	24	99
	1500	8	42	6	56	73	12	99
	3500	9	37	5	51	84	4	102
Total number of rats with a malignant tumor	0	6	39	12	57	23	13	38
	500	4	45	10	59	28	14	45
	1500	5	48	4	57	31	7	39
	3500	7	55	7	69	32	2	36

Source: Dow Chemical Company, 1980.

TABLE 5-13. TIME-TO-TUMOR, PALPABLE MASS, AND HISTOPATHOLOGY DATA FOR SALIVARY GLAND REGION SARCOMAS IN INDIVIDUAL MALE RATS EXPOSED TO METHYLENE CHLORIDE BY INHALATION FOR 2 YEARS

Animal number	Exposure group (ppm)	Month of study tumor first observed	Size of tumor when first observed (cm)	Month of study for necropsy	Size of tumor at necropsy (cm)	Histological diagnosis	Evidence of distant metastases
76-3301	0	15	5	15	7 x 6 x 4	Subcutaneous - undifferentiated sarcoma	None
76-3733	1500	16	3	17	5 x 4 x 2	Salivary gland - malignant schwannoma	None
76-3809	1500	Not observed	-	19	Not detected (slightly enlarged salivary gland)	Salivary gland - malignant schwannoma	None
76-3722	1500	24	1.5	24	4 x 3 x 2	Salivary gland - undifferentiated schwannoma	None
76-3743	1500	14	2	14	No size given (6 grams)	Subcutaneous - round cell sarcoma	None
76-3749	1500	18	3	20	5 x 4 x 4	Subcutaneous - round cell sarcoma	None
76-3583	3500	Not observed	-	24	Not detected	Salivary gland - fibrosarcoma	None
76-3698	3500	14	3	15	7 x 6 x 3	Salivary gland - carcinosarcoma	Yes
76-3580	3500	21	2	24	9 x 6 x 4.5	Subcutaneous - neurofibrosarcoma	Yes
76-3663	3500	20	2	21	No size given	Subcutaneous - fibrosarcoma	Yes

(continued on the following page)

TABLE 5-13. (continued)

Animal number	Exposure group (ppm)	Month of study tumor first observed	Size of tumor when first observed (cm)	Month of study for necropsy	Size of tumor at necropsy (cm)	Histological diagnosis	Evidence of distant metastases
76-3682	3500	21	6	21	7 x 4 x 4	Subcutaneous - undifferentiated round cell sarcoma	None
76-3578	3500	16	8	16	7 x 7 x 3.5	Subcutaneous - undiffferentiated sarcoma	None
76-3608	3500	18	2.5	18	6 x 4 x 3	Subcutaneous - pleomorphic sarcoma	None
76-3621	3500	17	4	18	5 x 5 x 3	Subcutaneous - pleomorphic sarcoma	None
76-3666	3500	15	3	16	8 x 6 x 4	Subcutaneous - pleomorphic sarcoma	Yes
76-3671	3500	Not Observed	-	14	3.5	Subcutaneous - neurofibrosarcoma	None
76-3597	3500	19	6	19	7 x 7 x 3.5	Subcutaneous - fibrosarcoma	None

Source: Dow Chemical Company, 1980.

TABLE 5-14. SUMMARY OF SALIVARY GLAND REGION SARCOMA INCIDENCE IN MALE RATS IN A 2-YEAR INHALATION STUDY WITH DICHLOROMETHANE

Dose	Incidence*	Fisher's exact test
0 ppm	1/93 (1%)	---
500 ppm	0/94 (0%)	---
1500 ppm	5/91 (5.5%)	(p = 0.10, N.S.)
3500 ppm	11/88 (12.5%)	(p = 0.002)

*Cochran-Armitage test for linear trend, $p < 0.0001$.

N.S. = Not significant.

Source: Dow Chemical Company, 1980.

5.3.3.1.2 Dow Chemical Company (1980) inhalation study in hamsters. A total of 866 Golden Syrian hamsters [Ela: Eng (syr) strain; Engle Laboratory Animals, Inc., Farmersburg, Indiana] (107 to 109/sex per exposure concentration) were exposed by inhalation to 0, 500, 1,500, and 3,500 ppm (0, 1735, 5205, and 12145 mg/m^3) of DCM. The materials and methods for experimental design are the same as mentioned previously in the rat portion of the Dow Chemical Company (1980) study. The body weights of the hamsters were 61 to 70 g when they were received. The hamsters were marked by unique toe clips for group identification and by ear punch for individual identification within the cages.

The mortality data for males and females are presented in Table 5-15. Female hamsters exposed to DCM at 3,500 ppm had a statistically significant decreased mortality from the 13th through the 24th mo of the study. Females exposed to 1,500 ppm also had statistically significant decreased mortality from the 20th through the 24th mo. This decreased mortality in females exposed to 3,500 and 1,500 ppm was considered to be exposure related. The remaining exposure groups of male (500, 1,500, and 3,500 ppm) and female (500 ppm) hamsters had no exposure-related differences in mortality. Some hamsters in each group had alopecia at 5.5 mo into the study, but this alopecia was secondary to a mange mite (Demodex species) infection. This parasite did not result in increased mortality or morbidity. No treatment-related differences were observed in the body weights of either males or females exposed to 500, 1,500, or 3,500 ppm of DCM.

TABLE 5-15. CUMULATIVE PERCENT MORTALITY OF HAMSTERS
2-YEAR DICHLOROMETHANE INHALATION STUDY

Month of study	DCM concentration, ppm							
	Male				Females			
	0	500	1,500	3,500	0	500	1,500	3,500
1	0	0	0	0	0.9	0	0.9	0
2	0	0	0	0	0.9	0	0.9	0
3	0	0	0	0	1.9	0.9	1.9	0.9
4	0	0	0	0.9	1.9	1.9	1.9	0.9
5	0	0.9	0	3.7	2.8	1.9	1.9	0.9
6	0	1.9	0	3.7	3.7	1.9	2.8	0.9
7	1.0	1.9	1.0	5.8	3.9	2.8	3.7	0.9
8	1.9	3.8	1.9	5.8	4.9	4.9	4.8	2.9
9	1.9	5.8	1.9	6.7	4.9	4.9	7.6	3.9
10	2.9	5.8	2.9	9.6	5.8	5.8	8.6	3.9
11	3.8	5.8	2.9	11.5	9.7	9.7	8.6	4.9
12	5.1*	6.7	3.9	12.5	13.3*	10.7	9.5	4.9
13	6.4	10.1	10.2	17.2†	22.6	11.7	13.3	4.9
14	10.6	14.1	13.3	24.2	26.9	16.3	15.0	7.1†
15	14.9	17.2	14.3	26.3	32.3	24.5	20.0	11.2†
16	23.4	18.2	17.3	33.3	36.6	26.5	24.0	15.3†
17	24.5	19.2	19.4	34.3	41.9	30.6	29.0	23.5†
18	31.9	24.2	24.5	37.4	52.7	32.7	32.0	27.6†
19	36.0	29.8	31.2	42.6	63.6	38.8	40.0	30.6†
20	37.1	43.6	41.9	55.3†	71.6	59.1	50.5	40.9†
21	41.6	51.1	54.8	60.6	80.7	63.4	55.8†	45.2†
22	56.2	61.7	68.8	69.1	88.6	70.0	61.1†	53.8†
23	61.8	63.8	75.3	74.5	94.3	82.8	68.4†	72.0†
24	82.0	78.7	88.2	85.1	100.0	91.4	75.8†	80.6†

*Five males and five females died due to food deprivation. These animals were subsequently deleted from mortality calculations.

†Significantly different from controls by Fisher's Exact Test, $P < 0.05$.

Source: Dow Chemical Company, 1980.

Based on the information available to the Carcinogen Assessment Group (CAG), it is very difficult to conclude whether the MTD was used. Dow Chemical Company has not submitted a 90-day dose-finding study, but a 30-day inhalation study has been reported in a letter from Dr. J. Burek to Dr. D. Singh, dated May 1, 1981.

"The study was conducted prior to the two-yr study, but has not been reported. CD-1 mice, Golden Syrian hamsters, Sprague-Dawley and CDF (F-344) rats were exposed to 0, 2,500, 5,000, or 8,000 ppm DCM vapor 6 hr/day, 5 days/week, for a total of 20, 19, 20 or 6-2/3 exposures, respectively, in 21-29 days. Body weight data was obtained throughout the study. Clinical chemistry parameters were measured. All animals underwent gross pathological examination at the termination of the experiment. The weights of the liver and kidneys were recorded from animals in the 0, 2,500, and 5,000 ppm groups, and organ/body weight ratios were calculated. Animals exposed to 8,000 ppm DCM exhibited anesthetic effects, increased blood urea nitrogen levels in Sprague-Dawley rats, and decreased body weights in rats. The animals exposed to 5,000 ppm showed slight anesthesia, decreased body weight in male rats, increased SGPT values in female mice and Sprague-Dawley rats and increased liver weights in female mice, hamsters and rats. Animals exposed to 2,500 ppm DCM appeared to scratch more than controls and therefore appeared to be affected, but showed no other effect attributable to exposure. The target organ in this study was the liver. Because of the results obtained, 8,000 and 5,000 ppm DCM were considered to be too high a dose level for the two-year study, and 2,500 ppm did not appear to have produced a severe enough response over the 30-day period. Therefore, concentrations of 3,500 ppm of DCM was [sic] chosen as the top dose for the two-year study."

Dow Chemical Company (1980) conducted a subchronic dose-finding study for a period of only 30 days rather than for the 90-day period that is usual in most animal bioassays. Further, body weight or mortality rate in the experimental group of hamsters does not decrease as compared to the controls. Hematologic determinations, serum clinical chemistry, urinalysis, bone marrow collection, and blood COHb determination were done in animals at the 6-, 12-, 18-, and 24-mo interim kills. No treatment-related effects were observed in any of the parameters evaluated in male or female hamsters after 6, 12, 18, or 24 mo of exposure to 500, 1,500, or 3,500 ppm of DCM, respectively.

Carboxyhemoglobin determinations were performed on the blood of male and female hamsters following 22 mo on test. Males and females exposed to DCM at 500, 1,500, or 3,500 ppm all had significantly elevated COHb values. There was a slight trend in a dose-response relationship in females, since the mean COHb value for those exposed to 500 ppm was 23.6%, while the values for females exposed to 1,500 or 3,500 ppm were 30.2% and 34.6%, respectively. However, an apparent dose-response relationship was not observed in males. Dow Chemical Company (1980) indicated that these data, when compared to those in the rat study, suggested that hamsters had a greater degree of metabolism of DCM to carbon monoxide. Furthermore, the apparent dose-response in females was surprising. As a result, additional hamsters were exposed to a single 6-hr exposure, and their COHb values were determined. There was no apparent sex difference, and the dose-response relationship observed in females after 22 months could not be verified. Since there was no dose-response relationship in male and female rats or male hamsters, and since the female hamsters exposed to a single 6-hr exposure did not show a dose-response relationship, the apparent trend for an exposure-related increase in female hamsters at 22 months may be a cumulative effect.

5.3.3.1.2.1 Gross and histopathologic observations of hamsters from the 6-, 12-, and 18-month interim kills. A variety of gross and histopathologic observations were recorded for hamsters that were sacrificed at the 6-, 12-, or 18-mo interim kills. Histopathologically, exposure-related differences were present, and consisted of decreased numbers of hamsters with amyloidosis of the liver, kidney, adrenals, thyroid, and spleen. A few animals in each group may or may not represent the trend of amyloidosis in males.

5.3.3.1.2.2 Gross and histopathologic observations of hamsters killed moribund or dying spontaneously during the study and those from terminal sacrifice (24-months).

Neoplastic and non-neoplastic observations--Gross and histopathologic examinations were conducted on all hamsters that died or were killed moribund during the study and on all surviving hamsters at the end of the study. The histopathologic observations for males and females are presented in the Dow Chemical Company report (1980, tables 124 and 127). The observations shown include all the neoplastic and non-neoplastic lesions recorded for these hamsters. Most observations were within the normal or expected range for Golden Syrian hamsters, as indicated by Dow Chemical Company. The female hamsters had increased incidences of lymphosarcoma in the experimental group. The incidences were 1/96, 6/95, 3/95, and 7/97 ($p = 0.033$) in the 0, 500, 1,500, and 3,500 ppm groups, respectively. A re-evaluation of lymphosarcoma data of female hamsters by the Carcinogen Assessment Group (CAG) resulted in the following incidences: 1/91, 6/92, 3/91, and 7/91 ($P = 0.032$) in the 0, 500, 1,500, and 3,500 ppm groups, respectively. The differences between the denominators above reflect the CAG's use of actual numbers of animals examined (which did not include animals that were severely cannibalized, autolyzed, or missing), whereas the Dow denominators included the total number of animals. Also, only a small number of mammary gland tissues were examined, and no lesions were found. Table 5-16 summarizes the total tumor data. The total number of hamsters with benign tumors increased significantly in females at 3,500 ppm; the total number of hamsters with malignant tumors increased significantly in males at 1,500 ppm.

In 1984, Burek et al. published the results of the Dow Chemical Company (1980) study.

5.3.3.1.3 Summary of the Dow Chemical Company (1980) rat and hamster inhalation studies. Based on all the data evaluated, the four following points are considered to be major findings in the rat and hamster studies:

1. Male rats exposed to 1,500 or 3,500 ppm appeared to have an increased number of sarcomas in the ventral midcervical area near the salivary glands. There were 1/93, 0/94, 5/91, and 11/88 ($P = 0.002$) sarcomas in male rats exposed to 0, 500, 1,500, or 3,500 ppm, respectively. Based on routine sections, special stains, and ultrastructural evaluations, these tumors appeared to be of mesenchymal cell origin; however, a myoepithelial cell origin of these cells could not be ruled out. These tumors had some areas that morphologically resembled one cell type (i.e., neurofibrosarcoma, fibrosarcoma), and still other tumors had cell types that were undifferentiated or pleomorphic. In some, one cell type was predominant, while in others, areas of all of the above cell types were present, depending on the area of the tumor examined. Furthermore, the origin of each of these tumors remains questionable. All appeared to be arising in the midcervical region, and all involved the salivary glands. Only two tumors were small enough to be localized within the salivary gland. The rest were larger tumors that clearly involved the salivary glands as well as adjacent tissues, and could have been growing either into or out of the salivary glands. However, they probably arose within the salivary glands based on the two localized small tumors described above.

Therefore, there was an apparent association between the increased incidence of sarcomas in the salivary gland region of male rats and prolonged exposure via inhalation to 1,500 or 3,500 ppm DCM. There were no salivary gland sarcomas in female rats or in hamsters of either sex. Further, it will be of interest to find out what kind of lesions are present or absent in the ongoing National Toxicology Program inhalation study.

TABLE 5-16. SUMMARY OF TOTAL TUMOR DATA FOR HAMSTERS ADMINISTERED DICHLOROMETHANE BY INHALATION FOR 2 YEARS

		Males				Females			
	Concentration (ppm)	Interim kills	Spontaneous deaths and killed moribund	Terminal kill	Cumulative totals	Interim kills	Spontaneous deaths and killed moribund	Terminal kill	Cumulative totals
Total number of hamsters during this period*	0	15	76	16	107	15	91	0	106
	500	10	74	20	104	10	88	4	102
	1500	10	82	11	103	10	81	10	101
	3500	15	78	14	107	14	82	9	105
Total number of hamsters with a tumor	0	4	18	3	25	2	17	-	19
	500	3	17	9	29	0	20	1	21
	1500	2	17	8	27	2	13	4	19
	3500	4	18	7	29	2	27	3	32†
Total number of hamsters with a benign tumor	0	4	14	2	20	2	11	-	13
	500	2	10	7	19	0	8	1	9
	1500	2	6	5	13	1	9	3	13
	3500	3	10	6	19	1	22	3	26†
Total number of hamsters with a malignant tumor	0	0	6	1	7	0	8	-	8
	500	1	8	4	13	0	13	0	13
	1500	0	12	3	15	1	4	2	7
	3500	1	8	1	10	1	9	0	10

*Does not include hamsters that escaped from their cages, or hamsters that were severely autolyzed, or severely cannibalized. Also, this total does not include 500 ppm or 1500 ppm male and female hamsters from the 6-month interim kill because no histopathology was done on these animals except for a liver special stain (Gomori's Prussian Blue Iron Reaction).

†Significantly different from controls when analyzed by Fisher's Exact Test, $P < 0.05$.

-Indicates none examined or not applicable.

Source: Dow Chemical Company, 1980.

2. Male and female rats exposed to DCM had increased numbers of benign mammary tumors as compared to control values. Female rats exposed to 500, 1,500, or 3,500 ppm of DCM had increased numbers of benign mammary tumors per tumor-bearing rat in comparison with the controls. The increase was evident in the palpable mass data and the gross necropsy findings, which were confirmed by the histopathologic examination. The total number of female rats with benign mammary tumors was not statistically increased in any exposure group (0, 500, 1,500, and 3,500 ppm groups had a total of 79, 81, 80, and 83 animals with benign mammary tumors, respectively). Sprague-Dawley rats have very high incidences of spontaneous mammary tumors. However, the total number of benign mammary tumors has increased in an exposure-related manner with 165/92 in the controls and 218/90, 245/92, and 287/95 in the females exposed to 500, 1,500, or 3,500 ppm, respectively. Expressed another way, the average number of benign mammary tumors per female rat increased from 1.7 in the controls, to 2.3 in those exposed to 500 ppm, to 2.6 in those exposed to 1,500 ppm, and to 3.0 in those exposed to 3,500 ppm. This increase was considered to be exposure-related and dose dependent.

A mammary tumor response was present in male rats also, but to a lesser extent than in females. The number of rats with benign mammary tumors in males exposed to 3,500 ppm increased but this increase was not statistically significant. The total number of benign mamary tumors in males exposed to 1,500 or 3,500 ppm increased slightly. As was the case in females, these effects in males exposed to 1,500 or 3,500 ppm were considered to be exposure-related.

There were no mammary gland tumors in male or female hamsters. Also only 28/92, 44/93, 30/94, and 27/93 mammary gland tissues were examined in the 0, 500, 1,500, and 3,500 ppm groups, respectively. Not a single lesion was recognized in the mammary gland tissues examined. The CAG feels that a greater number of hamster mammary gland tissues should have been examined to better evaluate the true incidence of mammary tumors.

3. There was an increased incidence of lymphosarcoma in female hamsters. The incidence was 1/96, 6/95, 3/95, and 7/97 ($p = 0.033$) in the 0-, 500-, 1,500-and 3,500-ppm groups, respectively Dow Chemical Company (1980). A re-evaluation of lymphosarcoma data of female hamsters by the CAG resulted in the following incidences: 1/91, 6/92, 3/91, and 7/91 ($P = 0.032$) in the 0, 500, 1,500, and 3,500 ppm groups, respectively. The differences between the

denominators above reflect the CAG's use of actual numbers of animals examined (which did not include animals that were severely cannibalized, autolyzed, or missing), whereas the Dow Chemical Company (1980) denominators included the total number of animals. Dow Chemical Company (1980) reported that the females exposed to 3,500 ppm had better survival (statistically significant) than the controls and thereby had a greater chance to develop these tumors. After correction for survival (1/39 versus 7/63) by the CAG, these data are not statistically significant ($P = 0.12$).

4. There appears to be a question as to whether the doses given the rat and hamster were at or near the MTD. The body weights of male rats increased particularly toward the latter part of the experiment, whereas the body weights of female rats were unaffected in any experimental group. Exposure to 3,500 ppm resulted in an increased mortality rate in female rats during the last 6 months, but the male rats were unaffected at any concentration. On the other hand, decreased mortality was observed in female hamsters exposed to 1,500 and 3,500 ppm, while mortality in male hamsters was unaffected at 500, 1,500, and 3,500 ppm based on only a 30-day rat and hamster inhalation (dose-finding) study. Based on this information, it is difficult to judge whether the animals were given a dose equal to the MTD.

5.3.3.1.4 Dow Chemical Company (1982) inhalation toxicity and oncogenicity study in rats. A 2-yr inhalation study of DCM with Sprague Dawley rats and Golden Syrian hamsters by Dow Chemical Company (1980) has been described earlier in this document. In that study, animals were exposed 6 hr/day, 5 days/week for 2 yr to DCM at 0, 500, 1,500, and 3,500 ppm. The carcinogenic response was positive in rats but negative in hamsters. In rats, the liver appeared to be the target organ affected by exposure. Hepatocellular vacuolization, consistent with fatty change, was observed in male and female rats inhaling 500, 1,500, or 3,500 ppm DCM. There was an increased incidence of multinucleated hepatocytes upon exposure to 500, 1,500, or 3,500 ppm, and an increased number of foci and areas of altered hepatocytes at 3,500 ppm in female rats. Benign mammary tumors were increased in male rats inhaling 1,500 or 3,500 ppm, and in female rats inhaling 500, 1,500, or 3,500 ppm DCM. Male rats exposed to 1,500 or 3,500 ppm DCM had an increased number of sarcomas in the region of the salivary gland. Female rats inhaling 3,500 ppm DCM had an increased mortality rate. Carboxyhemoglobin levels in the blood of rats exposed to DCM were higher than control levels; however, no differences were observed in COHb levels of animals inhaling DCM at 500 ppm versus animals inhaling it at 3,500 ppm. The objective of this second study was to further investigate the toxicity of DCM at concentrations far below those that may cause saturation of the metabolic processes in rats.

A total of 360 male and 492 female Sprague-Dawley rats (Spartan substrain, 6 to 8 weeks old) were used in this study. Groups of 90 rats/sex were exposed by inhalation to 0 (control), 50, 200, and 500 ppm (0, 173, 692, and 1,735 mg/m^3) DCM (technical grade, lot #TA 05038, with purity of at least 99.5 percent) 6 hr/day, 5 days/week, for 20 (males) or 24 mo (females). Occasionally an exposure was shorter than 6 hr, due to vapor generation or mechanical problem. In addition, 30 extra female rats, identified as 500/0, were exposed to 500 ppm DCM for the first 12 mo of the study and were housed as control rats for the duration of the study (last 12 mo). Another 30 female rats, identified as 0/500, were housed in the same manner as control rats for the first 12 mo of the study and were exposed to 500 ppm DCM for the remaining 12 mo of the study. To determine the rate of DNA synthesis in the liver, 18 female rats were included in each group. After 6, 12, 15, and 18 mo of exposure, five rats of each sex at each exposure level were sacrificed. In addition, five female rats from each of the 500/0 and 0/500 groups were sacrificed at the 18-mo interim necropsy.

Clinical laboratory tests for chemistry, plasma hormone levels, and DNA were made on interim sacrificed rats. Gross and microscopic examinations were made of animals at interim and terminal sacrifice, as well as of animals dying spontaneously and those that were killed moribund during the study.

As reported by the authors, the nominal and analytical concentrations of DCM in the chambers were in close agreement, indicating no detectable loss or decomposition of test material during vaporization. Approximately 2 mo after the initial exposure to DCM, symptoms consistent with sialodacryoadenitis (SDA virus) were observed in male and female rats in each experimental group, including control groups. The rats from all exposure groups appeared to be equally affected, and the symptoms were not apparent 3 weeks after the initial observation.

No significant difference in body weight gain was noticed in male rats, but the mean body weights of female rats at 50, 200, or 500 ppm were significantly higher throughout the study period in comparison with controls. Although the authors of this study consider this to be a reflection of biological variability, the CAG considered that the highest dose was not the MTD because the same strain of rat tolerated a dose of 3,500 ppm by inhalation in the previous study at the same laboratory (Dow Chemical Company, 1980).

No increase in mortality rate from that of the control group was observed in male or female rats. According to the authors, "...due to the high mortality rate in all groups of male rats, the terminal necropsy for male rats occurred during the 21st month of exposure to methylene chloride to ensure adequate numbers of surviving animals for pathologic evaluation." This is not consistent with the findings in Tables 5-17 and 5-18.

No significant effect on absolute or relative organ weight was seen in male or female rats. Blood COHb levels were significantly elevated ($P < 0.05$) above controls in all experimental groups of rats. Incorporation of ^3H-thymidine into hepatic DNA was unaffected in female rats at 6 and 12 mo. DNA synthesis in rats was not determined at 15 mo, as originally scheduled in the protocol, due to the number of mammary tumors observed in female rats at 200 and 500 ppm.

Results from the 6-, 12-, 15-, and 18-mo interim necropsies showed no definite exposure-related gross or histopathologic findings in male and female rats from any of the interim sacrifices. An exception was the interim sacrifice of female rats at 15 mo, where 1, 3, 4, and 5 females inhaling DCM at 0, 50, 200, or 500 ppm, respectively, had a focus or foci of altered cells in the liver. This effect was not apparent in female rats from 6-, 12-, or 18-mo interim sacrifices, nor was it apparent from rats dying spontaneously, killed moribund during the study, or terminally sacrificed.

There were no significant histological lesions observed in other organs, with the exception of the liver. Data on the liver lesions are given in Tables 5-19 and 5-20. In males, the incidence of hepatocellular vacuolization increased slightly (Table 5-19). The liver lesions in female rats were significantly increased for foci of altered cell, hepatocellular vacuolization, and multinucleated hepatocytes at 500 ppm (Table 5-20) as compared to controls. The significance of these alterations in the liver is not known. Further, the number of liver lesions appeared to be increased in the 500/0 group at terminal sacrifice, combined sacrifice, and death as compared to controls, but this was not the case in the 0/500 ppm group. There were no significant differences in any tumor type for liver, kidney, spleen, brain, salivary gland, lung, skin, pancreas, and mammary gland in male and female rats, with the exception of mammary gland tumors, which were significantly higher in females (Table 5-21).

TABLE 5-17. MONTHLY MORTALITY DATA FOR MALE RATS IN A 2-YEAR DICHLOROMETHANE INHALATION TOXICITY AND ONCOGENICITY STUDY (%)

Month of study	DCM concentration (ppm) 0	50	200	500
1	0*	0	0	0
2	0	0	0	0
3	0	1(1/90)	0	0
4	0	1(1/90)	1(1/90)	0
5	0	1(1/90)	1(1/90)	0
6	0	1(1/90)	1(1/90)	0
7	0	2(2/85)	2(2/85)	0
8	0	2(2/85)	4(3/85)	0
9	1(1/85)	4(3/85)	4(3/85)	0
10	1(1/85)	4(3/85)	4(3/85)	1(1/85)
11	4(3/85)	4(3/85)	5(4/85)	1(1/85)
12	4(3/85)	5(4/85)	7(6/85)	1(1/85)
13	5(4/80)	9(7/80)	11(9/80)	4(3/80)
14	15(12/80)	13(10/80)	14(11/80)	8(6/80)
15	20(16/80)	18(14/80)	23(18/80)	13(10/80)
16	33(25/75)	20(15/75)†	31(23/75)	23(17/75)
17	37(28/75)	37(28/75)	37(28/75)	25(19/75)
18	47(35/75)	45(34/75)	53(40/75)	41(31/75)
19	59(41/70)	64(45/70)	69(48/70)	50(35/70)
20	70(49/70)	70(49/70)	79(55/70)	66(46/70)
Terminal sacrifice	74(52/70)	73(51/70)	81(57/70)	73(51/70)

*Percent mortality (number dead/original number of animals minus animals sacrificed for an interim necropsy).

†Significantly different from control value by Fisher's Exact Test.

Source: Dow Chemical Company, 1982.

TABLE 5-18. MONTHLY MORTALITY DATA FOR FEMALE RATS IN A 2-YEAR DICHLOROMETHANE INHALATION TOXICITY AND ONCOGENICITY STUDY (%)

	DCM concentration (ppm)					
Month of study	0	50	200	500	0/500	500/0
1	0*	0	1(1/90)	0	0	0
2	0	0	1(1/90)	0	0	0
3	0	0	1(1/90)	0	0	0
4	0	0	1(1/90)	0	0	0
5	0	0	1(1/90)	0	0	0
6	0	0	1(1/85)	1(1/90)	0	0
7	0	0	1(1/85)	1(1/85)	0	0
8	0	0	2(2/85)	1(1/85)	0	0
9	1(1/85)	0	2(2/85)	1(1/85)	0	0
0	1(1/85)	0	2(2/85)	1(1/85)	0	0
1	2(2/85)	0	2(2/85)	1(1/85)	0	0
2	4(3/85)	2(2/85)	2(2/85)	4(3/85)	0	0
3	4(3/80)	5(4/80)	5(4/80)	6(5/80)	3(1/30)	0
4	5(4/80)	5(4/80)	5(4/80)	9(7/80)	3(1/30)	0
5	9(7/80)	9(7/80)	10(8/80)	15(12/80)	3(1/30)	0
6	12(9/75)	15(11/75)	13(10/75)	17(13/75)	7(2/30)	0†
7	15(11/75)	15(11/75)	15(11/75)	19(14/75)	7(2/30)	0†
8	19(14/75)	17(13/75)	21(16/75)	28(21/75)	17(5/30)	3(1/30)†
9	30(21/70)	27(19/70)	30(21/70)	37(26/70)	24(6/25)	8(1/25)†
10	36(25/70)	36(25/70)	34(24/70)	39(27/70)	32(8/25)	8(2/25)†
11	43(30/70)	46(32/70)	49(34/70)	47(33/70)	36(9/25)	16(4/25)†
12	53(37/70)	53(37/70)	54(38/70)	50(35/70)	48(12/25)	36(9/25)†
13	60(42/70)	63(44/70)	64(45/70)	54(38/70)	60(15/25)	48(12/25)†
Terminal sacrifice (after 24 months)	64(45/70)	76(35/70)	67(47/70)	61(43/70)	72(18/25)	52(13/25)

*Percent mortality (number dead/original number of animals minus animals sacrificed for an interim necropsy).

†Significantly different from control value by Fisher's Exact Test.

Source: Dow Chemical Company, 1982.

TABLE 5-19. NON-NEOPLASTIC LIVER LESIONS IN MALE RATS

	Exposure level (ppm)	Foci of altered cells	Hepatocellular vacuolization
Terminal kill	0	1/18	3/18
	50	3/19	9/19*
	200	5/13*	7/13*
	500	7/19*	8/19
Moribund and spontaneous death	0	6/52	19/52
	50	6/51	9/51
	200	6/57	14/57
	500	4/51	20/51
Combined	0	7/70	22/70
	50	9/70	18/70
	200	11/70	21/70
	500	11/70	28/70

*Fisher's Exact Test, $P < 0.05$.

Source: Dow Chemical Company, 1982.

TABLE 5-20. NON-NEOPLASTIC LIVER LESIONS IN FEMALE RATS

	Exposure level (ppm)	Foci of altered cells	Hepatocellular vacuolization	Multinucleated hepatocytes
Terminal kill	0	9/25	23/25	4/25
	50	5/17	15/17	4/17
	200	10/22	21/22	5/22
	500	17/27*	23/27	16/27*
	0/500	5/7	6/7	1/7
	500/0	8/12	12/12	6/12*
Moribund and spontaneous death	0	12/45	18/45	4/45
	50	12/53	27/53	2/53
	200	17/48	23/48	7/48
	500	14/43	30/43*	11/42*
	0/500	7/18	9/18	2/18
	500/0	5/13	4/13	3/13
Combined	0	21/70	41/70	8/70
	50	17/70	42/70	6/70
	200	27/70	44/70	12/70
	500	31/70	53/70*	27/70*
	0/500	12/25	15/25	3/25
	500/0	13/25	16/25	9/25*

*Fisher's Exact Test, $P < 0.05$.

0/500 = rats exposed to 500 ppm DCM for first 12 months.

500/0 = rats exposed to 500 ppm DCM for last 12 months.

Source: Dow Chemical Company, 1982.

TABLE 5-21. SUMMARY OF MAMMARY GLAND TUMORS IN FEMALE RATS

	Exposure level (ppm)	Number of rats	Moribund and spontaneous	Terminal kill	Cumulative
Rats with a benign mammary tumor (adenoma, fibroadenoma, or fibroma)	0	70	35	17	52
	50	70	43	15	58
	200	70	41	20	61*
	500	70	32	23	55
	0/500	25	17	6	23
	500/0	25	11	12*	23
Total number of benign mammary tumors (adenomas, fibroadenomas, and fibromas)	0	70	69	36	105
	50	70	97	36	133
	200	70	91	44	135
	500	70	68	79	147
	0/500	25	35	15	50
	500/0	25	27	33	60

*Significantly different from control when analyzed by Fisher's Exact Test, $P < 0.05$.

†Data could not be analyzed by Fisher's Exact Test.

0/500 = rats exposed to 500 ppm DCM for first 12 months.

500/0 = rats exposed to 500 ppm DCM for last 12 months.

Source: Dow Chemical Company, 1982.

In summary, there were significant increases in non-neoplastic liver lesions (i.e., hepatocellular vacuolization and multinucleated hepatocytes) in female rats at 500 ppm. There was an increase in benign mammary tumors (adenoma, fibroma, and fibroadenoma) in female rats. The number of benign mammary tumors/tumor-bearing rats observed in female rats was 2.0, 2.3, 2.2, and 2.7 in rats inhaling DCM at 0, 50, 200, and 500 ppm, respectively. Female rats of group 500/0 showed effects that were similar to rats exposed to 500 ppm for 24 months, but the 0/500 group did not differ from the control group.

In conclusion, the results of this study offer very limited evidence of the carcinogenicity of DCM. However, the highest dose in this study is half of the lowest doses in both the NTP gavage study (1982 draft) and the ongoing NTP inhalation study.

5.3.3.1.5 National Coffee Association (1982a,b) study in rats. On August 11, 1982, Hazelton Laboratories America, Inc., reported on a chronic study in Fischer 344 rats administered DCM in deionized drinking water for 24 mo. This study was sponsored by the National Coffee Association (NCA), and utilized 1,000 animals in 7 different dose groups or regimens (Table 5-22). The actual mean daily consumption levels of DCM in the drinking water were similar to the expected target levels (Table 5-23).

Interim sacrifices were performed at 26, 52, and 78 weeks of treatment with 5, 10, or 20 rats of each sex from each dose group, respectively, with the exception of the animals in Group 7 (the recovery group, which were only sacrificed with the remaining terminal animals at 104 weeks). The effects of compound administration were evaluated using the following criteria: survival, body weight gains, total food consumption, water consumption, clinical observations, ophthalmoscopic findings, clinical pathology, organ and tissue weights, and gross and microscopic pathology.

No compound-related findings were reported for either survival, clinical observations, opthalmoscopic findings, gross necropsy findings, or organ weight data. Throughout the study, small but significantly decreased body weight gains and water consumption were reported for both male and female rats in Groups 5, 6, and 7. Food consumption also decreased, but this criterion was only monitored for the first 13 weeks. These decreased effects were attributed to DCM administration. Low-magnitude statistical increases in mean hemotocrit, hemoglobin, and red blood cell counts were noted in both male and female animals of Groups 4, 5, and 6. In most cases, these were within the range of historical control values of Fischer 344 rats.

TABLE 5-22. GROUP ASSIGNMENT OF FISCHER 344 RATS ADMINISTERED DICHLOROMETHANE IN DEIONIZED DRINKING WATER FOR 24 MONTHS

Group number	Number of animals		Target dose, mg/kg/day
	Males	Females	
1. Control	85	85	0
2. Control	50	50	0
3. Low-dose	85	85	5
4. Mid-dose 1	85	85	50
5. Mid-dose 2	85	85	125
6. High-dose	85	85	250
7. High-dose/recovery (78 weeks/26 weeks)	25	25	250

Source: National Coffee Association, 1982a.

TABLE 5-23. MEAN DAILY CONSUMPTION OF DICHLOROMETHANE IN A 24-MONTH CHRONIC TOXICITY AND ONCOGENICITY STUDY IN FISCHER 344 RATS

Group	Target level, mg/kg/day	Males	Females
3	5	5.85	6.47
4	50	52.28	58.32
5	125	125.04	135.59
6	250	235.00	262.81
7	250*	232.13	268.72

*The designated recovery group (Group 7) mean is for the first 78 weeks only.

Source: National Coffee Association, 1982a.

Histopathological alterations were described in the livers of rats of both sexes in Groups 4, 5, 6, and 7. These changes consisted of an increased incidence of foci/areas of cellular alteration in Groups 4, 5, 6, and 7 and of fatty changes in Groups 5 and 6 after 78 and 104 weeks of treatment. The incidence of neoplastic nodules and/or hepatocellular carcinomas in female Fischer 344 rats (Table 5-24) was derived from the data presented in Volumes I-IV of the August 11, 1982, NCA report (National Coffee Association 1982a) and in the Addition to the Final NCA Report of November 5, 1982 (National Coffee Association 1982b). Male rats did not show an increased incidence of liver tumors in treated animals versus controls (Table 5-24). These statistically significant increases in the incidences of liver tumors in female rats were within the range of historical control values at this laboratory (Table 5-25), as presented in a letter from Dr. John Kirschman of General Foods to Dr. Dharm Singh of the CAG, dated February 17, 1983. Therefore, based on a review of the NCA study, DCM administered in deionized water at doses up to 250 mg/kg/day was borderline for carcinogenicity to Fischer 344 rats.

5.3.3.1.6 National Coffee Association (1983) study in mice. In December 1983, Hazelton Laboratories of America, Inc. reported on a chronic study in B6C3F1 mice administered DCM in deionized water for 24 mo. This study, sponsored by the National Coffee Association (NCA), utilized 1,000 mice (650 males and 350 females) in six different dose groups or regimens (Table 5-26). The actual mean daily consumption levels of DCM in drinking water were similar to the expected target levels (Table 5-27). Each animal was housed individually and identified by a unique number.

Adjusted survival for male mice at 104 weeks was 88.3% for Group 1, 76.6% for Group 2, 81.5% for Group 3, 81.0% for Group 4, 82.8% for Group 5, and 81.5% for Group 6. For female mice, adjusted survival at 104 weeks was 69.4% for Group 1, 78.0% for Group 2, 73.0% for Group 3, 84.0% for Group 4, 76.0% for Group 5, and 91.8% for Group 6. No compound-related significant differences between groups were found, but survival was slightly increased in Groups 4 and 6.

There were some exceptional clinical findings, i.e., convulsions characterized by quaking, extension of the head, and lowering of the pinnae of the ears (peyer reaction). In some cases the behavior progressed to full seizure. There was no correlation to any increased mortality. These convulsions were

TABLE 5-24. INCIDENCE OF HEPATOCELLULAR TUMORS IN MALE AND FEMALE FISCHER 344 RATS ADMINISTERED DICHLOROMETHANE IN DEIONIZED DRINKING WATER FOR 104 WEEKS

Diagnosis	Treatment group (mg/kg/day)						
	0	0	5	50	125	250	250 (Recovery)
				Male*			
Neoplastic nodule (NN)	4(4.7)	5(10)	2(2.4)	3(3.6)	3(3.5)	1(1.2)	4(16)
Hepatocellular carcinoma (HC)	2(2.4)	2(4)	0(0)	0(0)	0(0)	1(1.2)	0(0)
Combined NN and HC	6(7.1)	7(14)	2(2.4)	3(3.6)	3(3.5)	2(2.4)	4(16)
Total livers examined	85	50	85	84	85	85	25
				Female*			
Neoplastic nodule (NN)	0(0)	0(0)	1(1.2)	2(2.4)	1(1.2)	4(4.7)	2(8.0)
Hepatocellular carcinoma (HC)	0(0)	0(0)	0(0)	2(2.4)	0(0)	2(2.4)	0(0)
Combined NN and HC	0(0)	0(0)	1(1.2)	4(4.8)†	1(1.2)	6(7.1)†	2(8.0)†
Total livers examined	85	49	85	83	85	85	25

*Numbers in parentheses represent percent incidence for the particular lesion.

†Statistically significant ($P < 0.05$) by the Fisher's Exact Test using a combined control incidence of 0/134.

Source: National Coffee Association, 1982a,b.

TABLE 5-25. HISTORICAL CONTROL DATA OF LIVER NEOPLASIA IN FEMALE FISCHER 344 RATS AT HAZELTON LABORATORIES AMERICA, INC.

Study:	A		B	C	D	E	F		G	H	I	J	Study Totals
Group:	1	2	1	1	1	1	1	2	1	1	1	1	
LIVER (No. examined)	57	70	37	40	38	43	11	14	39	40	17	13	419
Neoplastic nodules	5	4	--	--	--	1	1	4	--	--	2	2	19
% Incidence	8.8	5.7	--	--	--	2.3	9.1	28.6	--	--	11.8	15.4	4.5
Hepatocellular carcinoma	--	--	--	--	1	1	1	1	--	--	--	1	5
%Incidence	--	--	--	--	2.6	2.3	9.1	7.1	--	--	--	7.8	1.2

-- = Not reported.

Source: Kirschman, 1983.

TABLE 5-26. GROUP ASSIGNMENT OF B6C3F1 MICE ADMINISTERED DICHLOROMETHANE IN DEIONIZED DRINKING WATER FOR 24 MONTHS

Group number	Number of animals		Target dose (mg/kg/day)
	Males	Females	
Control	60	50	0
Control	65	50	0
Low-dose	200	50	60
Mid-dose 1	100	50	125
Mid-dose 2	100	50	185
High-dose	125	50	250

Source: National Coffee Association, 1983.

TABLE 5-27. MEAN DAILY CONSUMPTION OF DICHLOROMETHANE IN A 24-MONTH CHRONIC TOXICITY AND ONCOGENICITY STUDY IN B6C3F1 MICE

Group	Target level (mg/kg/day)	Males	Females
		mg/kg/day ± S.D.	mg/kg/day ± S.D.
3	60	60.55 ± 7.680	59.46 ± 8.413
4	125	123.61 ± 14.356	118.19 ± 16.799
5	185	177.48 ± 19.214	172.41 ± 22.950
6	250	234.29 ± 26.495	237.76 ± 29.329

Source: National Coffee Association, 1983.

demonstrated in each group including the controls, at least once, and were noted during body weight procedure when animals were placed in pans. The occurrence of convulsions in B6C3F1 mice is presented in Table 5-28.

An increased number of Harderian gland neoplasms were also observed in males in Groups 4 and 6. The other groups were similar to the controls (Table 5-29). The increase in Groups 4 and 6 may be a result of normal biological variation as suggested by the sponsors.

No compound-related findings were observed for either survival, body weight changes, water consumption, clinical observation, leukocyte counts, and gross necropsy findings. Food consumption was not done in this experiment. Histomorphologic alterations of the liver were observed in both male and female mice in the high-dose group (containing greater amounts of oil Red O positive material). Hepatocellular lesions were observed in all groups of both sexes, including controls. The lesions in the treated female group were comparable to controls (Table 5-30). In males, there was also a statistically significant increase in the incidence of hepatocellular adenomas and/or carcinomas in Groups 4 and 5 and a borderline significant increase in Group 6 (the high-dose group).

Based on the results of this study, DCM administered in deionized water at doses up to 250 mg/kg/day can be considered borderline for carcinogenicity to B6C3F1 male mice.

5.3.3.1.7 National Toxicology Program (1982 draft) gavage study in rats and mice. The National Toxicology Program (NTP) conducted a 2-yr carcinogenesis bioassay of food-grade DCM and reported on their results in a draft technical report dated September 22, 1982 (National Toxicology Program, 1982). Dichloromethane was administered in corn oil by gavage to male and female Fischer 344/N rats and B6C3F1 mice. The DCM was more than 99.5% pure, with vapor phase chromatographic analysis detecting the presence of vinylidene chloride and trans-dichloroethylene up to 0.4%. A 13-week dose finding study was conducted to evaluate the toxicity of the compound. Based on survival, body weight gain, and histopathological examination, doses of 500 and 1,000 mg/kg by gavage were selected for male and female rats and mice for the 2-yr study.

The NTP announced on July 25, 1983 that the draft NTP report would not be issued as a final report due to discrepancies in experimental data that compromise a clear interpretation. NTP further allowed that pending the results

TABLE 5-28. OCCURRENCE OF CONVULSIONS IN MALE AND FEMALE B6C3F1 MICE

Males						Females					
1	2	3	4	5	6	1	2	3	4	5	6
Percent with convulsions at least once											
86.8	89.8	80.4	74.1	76.8	77.2	100.0	89.7	90.7	88.1	94.7	91.1
Mean frequency of occurrence											
5.4	6.3	4.1	4.3	5.2	5.0	8.6	7.6	8.0	8.0	7.1	8.3

Source: National Coffee Association, 1983.

TABLE 5-29. NUMBER OF MALE MICE WITH HARDERIAN GLAND NEOPLASMS

Group: Number examined:	1 60	2 65	3 200	4 100	5 99	6 121
Harderian gland						
Adenoma	3	2	13	9	5	11
Carcinoma	0	2	0	0	0	0
Percent						
Adenoma	5	3	7	9	5	9
Carcinoma	0	3	0	0	0	0
Combined	5	6	7	9	5	9

Source: National Coffee Association, 1983.

TABLE 5-30. INCIDENCE OF PROLIFERATIVE HEPATOCELLULAR LESIONS IN MALE AND FEMALE B6C3F1 MICE ADMINISTERED DICHLOROMETHANE IN DEIONIZED DRINKING WATER FOR 104 WEEKS

	Treatment group					
Group: Number examined:	1 60	2 65	3 200	4 100	5 99	6 125
			Male*			
Hyperplasia	4 (7%)	6 (9%)	14 (7%)	4 (4%)	10 (10%)	13 (10%)
Hepatocellular adenoma	6 (10%)	4 (6%)	20 (10%)	14 (14%)	14 (14%)	15 (12%)
Hepatocellular carcinoma	5 (8%)	9 (14%)	33 (17%)	18 (18%)	17 (17%)	23 (18%)
Adenoma and/or carcinoma	11 (18%)	13 (20%)	51 (26%)	30 (30%)	31 (31%)	35 (28%)
Fisher Exact Test			0.12	0.042†	0.027†	0.068
			Female*			
Hyperplasia	6 (12%)	0 (0%)	3 (3%)	1 (2%)	0 (0%)	3 (6%)
Hepatocellular adenoma	1 (2%)	2 (4%)	3 (3%)	1 (2%)	2 (4%)	1 (2%)
Hepatocellular carcinoma	2 (4%)	1 (2%)	1 (1%)	1 (2%)	3 (6%)	2 (4%)
Adenoma and/or carcinoma	3 (6%)	3 (6%)	4 (4%)	2 (4%)	5 (10%)	3 (6%)

*Numbers in parentheses represent percent incidence for the particular lesion.

†Statistically significant ($P < 0.05$) using a combined control incidence of 24/125.

Source: National Coffee Association, 1983.

of an in-depth audit, select and relevant information from these gavage studies might be incorporated into the future draft technical report for DCM inhalation studies.

5.3.3.1.8 Theiss et al. (1977). A pulmonary tumor bioassay in mice was reported by Theiss et al. (1977). Groups of 20 male strain A mice were injected intraperitoneally three times a week with 0, 160, 400, or 800 mg/kg DCM for a total of 16 or 17 injections. Mice were sacrificed 24 weeks after the first injection, and the lungs were examined under a dissecting microscope for surface adenomas. Some adenomas were confirmed by histology.

Tumors were found at all three dose levels; however, due to poor survival and the small number of animals, the increase in tumors did not reach statistical significance at the two highest dose levels (Table 5-31). At the lowest dose, a highly significant increase in the number of tumors was observed (P = 0.013). Therefore, this study was marginally positive for carcinogenicity.

TABLE 5-31. PULMONARY TUMOR BIOASSAY IN STRAIN A MICE

Dose (mg/kg)	Total dose given	No. of mice at beginning	No. of mice examined for tumors	Tumors/ mouse	Significance*
0	0	20	15	0.27	--
60	2,720	20	18	0.94	<0.013
400	6,800	20	5	0.80	>0.1
800	12,800	20	12	0.50	>0.1

Source: Adapted from Thiess et al., 1977.

*The test of significance used is the exact test of ratio of two Poisson parameters.

5.3.3.1.9 Heppel et al. (1944). Heppel et al. (1944) exposed dogs, rabbits, guinea pigs, and rats to DCM by inhalation at levels of 5,000 ppm (17,350 mg/m^3) for 7 hr/day and 10,000 ppm (34,700 mg/m^3) for 4 hr/day, 5 days/week, for 6 months. No tumors developed in any animals.

5.3.3.1.10 MacEwen et al. (1972). MacEwen et al. (1972) exposed dogs to DCM by inhalation at 500 ppm (1,735 mg/m^3) for 14 weeks; no tumors were reported, but edema of the meninges of the brain occurred. Neither this study nor the

Heppel et al. (1944) study could have detected a carcinogenic response because of the shortness of the observation times and the fact that these studies were not originally designed to test for carcinogenicity.

5.3.3.1.11 Other animal studies in progress. The National Toxicology Program sponsored a 2-yr inhalation study in male and female Fischer 344/N rats and B6C3F1 mice at exposure concentrations of 0, 1,000, 2,000, and 4,000 ppm (0, 3,470, 6,940, and 13,880 mg/m^3) for rats and 0, 2,000, and 4,000 ppm (0, 6,940, and 13,880 mg/m^3) for mice. The animals have been sacrificed (April 1983), but the pathology report is not yet available.

5.3.3.1.12 Cell transformation studies. Price et al. (1978) exposed Fischer rat embryo cell cultures (F1706, subculture 108) to DCM liquid at concentrations of 1.6 x 10^2 and 1.6 x 10^3 μM for 48 hr. Dichloromethane was diluted with growth medium to yield the appropriate doses. The DCM sample, obtained from the Fisher Scientific Company, was ≥99.9% pure. The cells were grown in Eagles minimum essential medium in Earle's salts supplemented with 10 percent fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 μg penicillin, and 100 μg streptomycin/ml. Quadruplicate cultures were treated at 50 percent confluency with each dose. After treatment, cells were cultured in growth medium alone at 37°C. Transformation of cells treated with either dose level of DCM was observed by 23 and 30 days of incubation, and was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation. There was no transformation of cells grown in medium alone or in the presence of a 1:1,000 acetone concentration, even after a subculture. Twenty and 27 microscopic foci per three dishes with the low and high DCM doses respectively, were found in dishes inoculated with 50,000 cells from cultures treated four subcultures earlier and held for 4 weeks at 37°C in a humidified CO$_2$ incubator before staining.

Subcutaneous injection of cells treated with 1.6 x 10^2 μM DCM five subcultures earlier produced local fibrosarcomas in 5/5 newborn Fischer 344 rats within 60 days following treatment. The ability of cells grown in growth medium alone to induce local fibrosarcomas was not determined; however, negative responses were obtained with cells grown in the presence of a 1:1,000 concentration of acetone. Exposure of cells to 3.7 x 10^{-1} μM 3-methylcholanthrene produced 124 microscopic foci per three dishes in the inoculation test described above by 37 days of incubation, and local fibrosarcomas in 12/12 rats by 27 days following subcutaneous injection of cells. The exposure of 3-methylcholanthrene was attained by initial dilution in acetone to 1 mg/ml followed by further dilution in growth medium to 0.1 µg/ml (personal communication from Dr. P. J. Price).

Dr. Price wrote a letter to the CAG dated Nov. 14, 1980, saying that "the analysis of methylene chloride showed a purity of 99.9 percent. The original study was done in quadruplicate and in each case the Fischer rat cells were transformed. Since the publication, the same batch of methylene chloride was sent to Andy Sivak at Arthur D. Little to be run against the Kakunago clone A31 of BALB/c 3T3. It did not transform his cells. We then repeated the study in Fischer rat cells and at the same time tested methylene chloride sent to us by the National Coffee Producers Association. The test was run in triplicate. The Fisher methylene chloride again transformed the cells, while the National Coffee Producers' (supplied by Diamond Shamrock) was negative." The differing responses in the two experiments may have been due to differences in the levels of impurities present in the samples used. The chemical compositions of DCM samples from different suppliers are given in Table 5-32.

The Fischer rat embryo cell line contains the genome of the Rauscher leukemia virus, but there is no basis for minimizing the positive results. Since the mode of action of DCM is not known, this transformation may be due to activation of the virus.

5.3.3.2 Epidemiologic Studies

5.3.3.2.1 Friedlander et al. (1978), Hearne and Friedlander (1981). Friedlander et al. (1978) performed mortality analyses of male Eastman-Kodak employees exposed to low levels of DCM. This study was updated by Hearne and Friedlander (1981). Measurements from 1959 to 1975 ranged from 30 to 120 ppm (104 to 416 mg/m^3). Both the original 1978 analysis and the 1981 update found no increase in neoplasms, heart disease, or any other cause of death in comparison with the two control groups, which were composed of other Kodak employees and of New York State males. The population was relatively stable and the workers were rotated throughout the work area, and thus exposure was averaged among all the workers. Dichloromethane had been used for 30 years as the primary solvent in this Eastman-Kodak operation.

Two separate mortality analyses were done. In the earlier paper, one approach used the proportionate mortality ratio to examine 334 deaths of DCM-exposed workers during 1956 to 1976. Seventy-one neoplasms were found; 73 were expected based on other Kodak employee mortality ratios. Furthermore, no single site was over-represented.

TABLE 5-32. CHEMICAL COMPOSITIONS OF DICHLOROMETHANE SAMPLES (ppm)[a]

	Fisher D-123[b] Lot 761542	Diamond Shamrock[c]	Dow[d]
Methyl chloride	0.5	<1	<1
Vinyl chloride	<0.1	<1	<1
Ethyl chloride	0.8	<1	3
Vinylidine chloride	329	<1	33
Carbon tetrachloride	3.6	<1	<10
Chloroform	<0.1	26	<5
Trichloroethylene	<0.1	<1	<1
1,2-Dichlorethylene	369	20	86
Methyl bromide	---	---	11
Cyclohexane	---	300	305

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[a] Presented by Drs. Sivak and Kirschman at the Science Advisory Board Meeting, Sept. 4-5, 1980.

[b] Fisher sample used by Dr. P. Price in two series of cell transformation studies.

[c] This material is under test in NCA's chronic rat study on DCM in drinking water. It was also used in Dr. Price's follow-up study.

[d] This material, also analyzed by NCI, with the same results as given by Drs. Sivak and Kirschman, was used in the following tests:

NCA's 90-day studies in rats and mice
Dr. Sivak's neoplastic transformation assay
Dr. P. Price's follow-up series of cell transformation tests
NCI's chronic bioassay studies in rats and mice presently under way

A second approach, included in the first paper and used exclusively in the update, was a cohort mortality study of all 751 employees who were in the DCM work area in 1964. The results of the update are shown in Tables 5-33 and 5-34, taken from that paper. There were 110 deaths during the 16-year follow-up (retrospective). Two control groups were used: other Kodak employees, and New York State males. The expected numbers of deaths in the exposed groups based upon the control group experiences were 105 and 168, respectively. The differences between the observed and the expected deaths based on the controls are either not statistically significant (other Kodak employees) or the expected deaths based on New York State males are significantly increased over the observed numbers.

The results show that malignant neoplasms accounted for 24 of the 110 deaths in the study cohort, which was less than the 28.6 or 38.5 expected malignancies based on the control data. Nine of these 24 deaths were from respiratory cancer (7.6 and 13.6 were expected based on the control groups) and seven were from cancers of the digestive organs (less than expected). Only the two deaths associated with brain or nervous tissue represented a higher than expected total (SMR = 169 and SMR = 227 versus two control groups), but these SMRs (standard mortality ratios) were not statistically significant.

Further stratification of the cohort focused on the 252 males with 20 years or more of exposure who were employed in 1964. In this group 59 deaths occurred: 13 were due to malignant neoplasms (17.8 and 24.7 expected based on the two control groups) and 32 were due to circulatory diseases (37.9 and 59.7 expected).

A further analysis of the 252 males shows that this cohort had a median age of approximately 54 years in 1964. With this group the more common cancers would have to be markedly increased for there to be a reasonable probability of detecting the increase. For example, following the cohort for 16 years, cancer mortality at this age would require 10 deaths from respiratory cancer to detect a significant result at the $P = 0.05$ level. This represents an increase of at least 100 percent over that expected, the expected probability least 100 percent over that expected, the expected probability of lung cancer death for this cohort being 0.018 over the 16 years, based on other Kodak employees' rates. The statistical power to detect 100-percent increases (at $\alpha = 0.05$, one-sided) is about 95 percent for all malignancies and 45 percent for respiratory cancer deaths. The remaining 499 males with less than 20 years

TABLE 5-33. OBSERVED AND EXPECTED DEATHS, 1964-1980, 1964 HOURLY MALE DICHLOROMETHANE COHORTS FROM KODAK

Diagnostic group	Cohort exposed minimum 20 years			Total 1964 CH_2Cl_2 cohort		
	No. observed	KP[a] no. expected	NYS[b] no. expected	No. observed	KP[a] no. expected	NYS[b] no. expected
Malignant neoplasms	13	17.77	24.72[b]	24	28.64	38.54[c]
Circulatory diseases	32	37.91	59.73[c]	62	59.11	90.90[c]
Ischemic heart disease	27	27.34	45.50[c]	48	43.40	69.54[c]
Other circulatory	5	10.57	14.23	14	15.71	21.36
Respiratory diseases	5	3.64	7.11	8	5.17	10.32
Accidents	2	2.35	3.90	6	5.53	9.80
All other causes	7	4.27	10.60	10	6.63	18.44
All causes	59	65.94	106.06[c]	110	105.08	168.00[c]

[a] Based on age-sex specific mortality of hourly men, rest of Kodak Park, 1964-1976.

[b] Based on age-sex specific mortality New York State (excluding New York City) for the years 1965, 1970, 1975.

[c] Observed-expected differences significant ($P \leq 0.05$).

Source: Hearne and Friedlander, 1981.

TABLE 5-34. MALIGNANT NEOPLASMS, OBSERVED AND EXPECTED DEATHS, 1964-1980, 1964 HOURLY MALE DICHLOROMETHANE COHORTS FROM KODAK

	Cohort exposed minimum 20 years			Total 1964 CH_2Cl_2 cohort		
Diagnostic Group	No. observed	KP[a] no. expected	NYS[b] no. expected	No. observed	KP[a] no. expected	NYS[b] no. expected
Digestive organs	5	7.08	7.73	7	10.93	11.68
Pancreas	2	1.34	1.48	3	2.36	2.24
Other	3	5.74	6.25	4	8.57	9.44
Respiratory	4	4.60	8.67[c]	9	7.56	13.62
Genitourinary	0	2.19	3.48	1	3.23	4.94
Brain and other nervous	1	0.62	0.44	2	1.18	0.88
Lymphatic and hematopoietic	0	1.44	2.00	1	2.68	3.45
Other malignant neoplasms	3	1.84	2.40	4	3.06	3.97
Total malignant neoplasms	13	17.77	24.72[c]	24	28.64	38.54[c]

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[a] Based on age-sex specific mortality of hourly men, rest of Kodak Park, 1964-1976.

[b] Based on age-sex specific mortality New York State (excluding New York City) for the years 1965, 1970, 1975.

[c] Observed-expected differences significant ($P \leq 0.05$).

Source: Hearne and Friedlander, 1981.

exposure were significantly younger (median age about 36 years), and a follow-up of this cohort for 16 years mortality might fail to detect even a moderate effect, since expected cancer mortality in this age group is so low.

5.3.3.2.2 Ott et al. (1983a,b,c,d,e). Ott et al. (1983a,b,c,d,e) reported the results of a health evaluation of employees of one fiber production plant who were exposed to DCM as part of a solvent mixture consisting of this substance plus methanol and also acetone in a separate container. A second fiber production plant utilizing only acetone, but similar to the first in other respects, was selected as a referent population. The investigation focused primarily on health effects occurring to the cardiovascular system stemming from the metabolism of DCM to COHb in the body. In addition to a retrospective cohort mortality study, the authors examined several other health end points in the still-employed group. These involved clinical evaluations, electrocardiographic monitoring, metabolism tests, and evaluation of oxygen half-saturation pressures.

Environmental exposure in the plant varied from 140 ppm (486 mg/m^3) in areas of low DCM use to 475 ppm (1,748 mg/m^3) in areas of high DCM use, based on an 8-hr time-weighted average (TWA). Methanol was present in smaller quantities by a factor of ten, while acetone ranged from 100 to 1,000 ppm (347 to 3,470 mg/m^3) TWA in both plants (Ott et al., 1983a). Industrial hygiene surveys were conducted from September 1977 to February 1978.

To qualify for inclusion in the cohort, production employees (both men and women) had to have worked a minimum of 3 months in the preparation or extrusion areas of either plant during January 1, 1954, to January 1, 1977. In the DCM plant, both cellulose diacetate (acetate) and cellulose triacetate (CTA) fibers were made side by side. Although acetone was present in both plants, it was the solvent of choice for making acetate fibers in the referent plant. The exposed cohort consisted of 1,271 persons versus 948 persons in the referent plant, as follows:

		DCM plant	plant
White	men	487	696
	women	615	248
Nonwhite	men	64	1
	women	105	3
Total		1271	948

Persons were followed through June of 1977. The authors noted that vital status was not available for 226 (18 percent) of the DCM-exposed cohort and 112 (12 percent) of the companion plant. The authors commented that few deaths would be added from this last group based on "previous experience with the social security follow-up mechanism." Such a statement is subject to question, however, without knowing the age distribution of this group.

The authors found no excessive mortality from any cause in the "exposed" or the "referent" population, either when the group with unknown vital status was presumed lost to follow-up at the time lost, or was presumed alive until June of 1977; i.e., person-years would cease accumulating at the time the person was lost to follow-up in the former instance, but in the latter case, person-years would continue to accumulate until the end of the follow-up period, as if the individual were still alive. Altogether in the exposed category for white men, 37 observed deaths out of 487 were seen versus 34.9 expected based on the latter definition, or 30.4 expected based on the former definition regarding the group with unknown vital status. For white women, only 11 deaths were observed versus either 15.9 or 13.5, depending upon the choice of the first or second definition above. For malignant neoplasms in white men, there were five observed deaths versus either 6.3 or 5.6 expected. In white women, two observed deaths were seen, versus either 5.2 or 4.5 expected. No cancer deaths were reported in nonwhite males and females, probably due to the small size of these select subgroups. Since the authors were mainly interested in cardiovascular effects, they examined further only ischemic heart disease in terms of duration of exposure and length of follow-up. Even when one considers only individuals with a minimum of 10 years of exposure who were followed for a minimum of 10 years, only 2 male deaths were observed versus 1.7 expected based on this cause. Ischemic heart disease was not observed as a cause of death in women. No corresponding data are available for any form of cancer by latency or duration of employment.

Several qualifications limit the possible use of this study as a sensitive indicator of mortality. Foremost among these is the relatively low and unusual distribution of mortality among the members of the cohort. Because of the excessive numbers of observed and expected deaths from external causes compared to the numbers of observed and expected deaths from malignant neoplasms, and the fact that deaths from accidents are the leading cause of death in young males nationwide, it is quite likely that the cohort is a relatively youthful group. This is further evidenced by the surprisingly few deaths observed overall compared to the size of the cohort--less than 10 percent in all instances (white men, nonwhite men, and white females). No age breakdown was provided to evaluate this observation.

Additionally, only 310 of the exposed males have had a chance to be followed at least 17.5 years. Some 241 exposed males entered the cohort after 1960 and could not possibly have been followed 17.5 years. Because of the 15- to 20-year latency period involved with most human cancers, cancer effects attributable to DCM exposure would probably not have been expected to manifest themselves prior to the 17th year. Thus, the power of this study to detect a statistically significant elevated risk of cancer (as well as ischemic heart disease) is low.

Another possible problem deals with the extent of follow-up of the cohort. Almost 18 percent (226) of the exposed cohort was lost to follow-up as of June 1977. Although the authors discount that as unimportant, it should be of concern that if the ages of the lost-to-follow-up group are relatively advanced, the likelihood is great that enhanced mortality will be in the higher age groups. Whenever extraordinary means are employed to determine the vital status of a subgroup of the cohort for which all other methods of follow-up have failed, the residual deaths found as a result of this endeavor are usually overly represented by sudden deaths due to heart failure or accidents. The opportunity for leaving a record of their deaths is minimized because of the nature of the deaths; hence, it becomes less likely that the vital status can be determined.

Still another problem with this study is the "healthy worker" effect. In most studies of this kind, workers at the time of their employment and shortly thereafter are generally somewhat healthier than the population from whence they came. Ill or infirm persons do not usually choose jobs that may be detrimental to their health. This tendency usually results in as much as a 20 percent deficit of mortality compared to that expected.

In summary, this study is inadequate to assess cancer mortality in the described cohort for the reasons stated. The study results do not exclude the possibility of increased health risk in the study population. The study focuses mainly on heart disease as a consequence of DCM exposure.

5.3.3.3 Quantitative Estimation. This quantitative section deals with the unit risk for DCM in air and water and the potency of DCM relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the incremental lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 $\mu g/m^3$ of the agent in the air they breathe or to a concentration of 1 μg/l in the water they drink. This calculation provides a quantitative estimation of the impact of the agent as a carcinogen. Unit risk estimates are used to compare the carcinogenic potency of several agents with each other and to give a crude indication of the population risk that might be associated with air or water exposure to these agents, if the actual exposures are known.

5.3.3.3.1 Procedures for the determination of unit risk for animals. The data used for the quantitative estimate are taken from one or both of the following: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, with an incidence determined by an extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time, the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA and are mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). Also, some evidence from animal experiments is consistent with the linear non-threshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large scale ED_{01} study at the National Center for Toxicological Research, and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation in the low-dose region of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk; i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95 percent confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

5.3.3.3.1.1 Description of the low-dose animal extrapolation model. Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\ [-(q_0 + q_1 d + q_2 d + q_2 d^2 + \ldots + q_k d^k]$$

where

$$q_i \geq 0,\ i = 0, 1, 2, \ldots, k.$$

Equivalently,

$$P_t(d) = 1 - \exp\ [-(q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has the approximate form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper limit, q_1^*, is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value, q_1^*, for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk, $P_t(d)$, is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted, and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i\ (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

5.3.3.3.1.2 Selection of data. For some chemicals, several studies using different animal species, strains, and sexes and run at several doses and different routes of exposure are available. A choice must be made as to which of the data sets from several studies to use in the model. The procedures used in evaluating these data are consistent with the approach of making a maximum likely-risk estimate. They are listed as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is significantly higher statistically than the control for at least one test dose level or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^* is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

5.3.3.3.1.3 Calculation of human equivalent dosages. It is appropriate to correct for metabolism differences between species and absorption factors via different routes of administration. Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since the surface area is approximately proportional to the two-thirds power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner. Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal.

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

Inhalation--When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas that reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

Case 1--Agents that are in the form of particulate matter of virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportionally to the breathing rate. In this case the exposure in mg/day may be expressed as

$$m = I \times v \times r$$

where I = inhalation rate per day in m^3, $v = mg/m^3$ of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations of the Federation of American Societies for Experimental Biology (FASEB, 1974) that 25-g mice breathe 34.5 liters/day and 113 g-rats breathe 105 liters/day. For mice and rats of other weights W (in kilograms), the surface area proportionality can be used to find breathing rates in m^3/day, as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}.$$

For humans, the value of 20 m^3/day* is adopted as a standard breathing rate (International Commission on Radiological Protection, 1977).

The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake per kilogram per day, $i = I/W$, based upon the previously stated relationships, are tabulated as follows:

Species	W	i = I/W
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

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*From "Recommendation of International Commission on Radiological Protection," page 9. The average breathing rate is 10^7 cm^3 per 8-hour workday and 2×10^7 cm^3 in 24 hours.

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr.$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

Case 2--The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr.$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or $\mu g/m^3$ in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al., 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

5.3.3.3.1.4 Calculation of the unit risk from animal studies. The 95 percent upper-limit risk associated with d $mg/kg^{2/3}$/day is obtained from GLOBAL79, and for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1^*d)$. A "unit risk" in units X is the risk corresponding to an exposure of X = 1. This value is estimated by finding the number of $mg/kg^{2/3}$/day that corresponds to one unit of X and substituting this value into the relationship expressed above. Thus, for example, if X is in units of $\mu g/m^3$ in the air, we have for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ $\mu g/kg^{2/3}$ per day, and for case 2, $d = 1$, when $\mu g/m^3$ is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, the following calculation may be used:

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3.$$

Note that an equivalent method of calculating unit risk would be to use mg/kg/day for the animal exposures, and then to increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, k$$

and to use mg/kg/day equivalents for the unit risk values. In the section calculating the unit risk for animals, the final q_1^* will always be the upper-limit potency estimate for human risk based on animal data.

5.3.3.3.1.5 Interpretation of quantitative estimates. For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution, diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably inhalation.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, or evaluating the adequacy of technology-based controls. However, with present technology, only imprecise estimations are possible concerning cancer risks to humans at low levels of exposure. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk, and while the true risk is probably not much more than the estimated risk, it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded, therefore, as accurate representations of the true cancer risks even when the exposures are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

5.3.3.3.1.6 Alternative methodological approaches. Methods used by the CAG for quantitative assessment are consistently conservative, i.e., they tend toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the linear non-threshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models, the one-hit, Probit, and Weibull models, have not been used by the CAG in the following analysis, but are included for comparison in the appendix. The CAG's position is that, given the limited data available from these animal bioassays, especially at the high-dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels and that the risk estimates obtained by use of the linear non-threshold model represent plausible upper limits only.

Extrapolation from animals to humans could also be done on the basis of relative weight rather than on the basis of relative surface area. Although the latter approach, used here, has more justification in terms of human pharmacological responses, it is not yet clear which of the two approaches is more appropriate for the assessment of carcinogenicity. In the absence of information on this point, it seems appropriate to use the more conservative basis for extrapolation, the relative surface area.

5.3.3.3.2 Unit risk potency estimates and relative potency. Neither of the two available epidemiologic studies provided positive results from which to derive a quantitative risk estimate for DCM exposure. With respect to animal data, three chronic studies are relevant to this discussion: two inhalation studies and one drinking water study. These are summarized in Table 5-35. In

TABLE 5-35. SELECTED DICHLOROMETHANE CHRONIC ANIMAL STUDIES

Author	Animal species and strains	Numbers	Routes	Doses, ppm	Results
Dow (1980)	Sprague-Dawley rats	95 rats/sex/dose	Inhalation 6/hr/day 5 days/week for 2 years	0, 500, 1500, and 3500	Salivary gland sarcoma in males
Dow (1980)	Syrian hamsters	107-109 hamsters sex/dose	Inhalation 6 hr/day 5 days/week for 2 years	0, 500, 1500, and 3500	--
Dow (1982)	Sprague-Dawley rats	90 rats/sex/dose	Inhalation 6 hr/day 5 days/week for 20 (males) or 24 (females) months	0, 50, 200, and 500	--
NCA (1982a,b)	Fischer 344 rats	85 rats/sex/dose; 135 male controls; 134 female controls	Deionized drinking water for 24 months	0, 5, 50, 125, 250, 250 (recovery)	Borderline neoplastic nodule response of the liver in females

the two inhalation studies (Dow Chemical Company 1980, 1982) only the salivary gland regions in male Sprague-Dawley rats in the 1980 study showed statistically significant increased cancers. These results are discussed in Section 5.3.3.1.1.2 and are presented in Tables 5-12 through 5-14. In the drinking water study (NCA 1982), the increase over untreated controls in combined neoplastic nodules and hepatocellular carcinomas in female rats was the only statistically significant finding (Table 5-24). However, when compared with historical controls, these results lose their statistical significance.

5.3.3.3.2.1 Unit risk ($\mu g/m^3$) for inhalation studies. The data used for estimates of the unit risk for inhalation are presented in Table 5-36, which shows the positive salivary gland region sarcomas for the inhalation bioassay. Exposure was 6 hr/day, 5 days/week, for 2 years. Equivalent dosages were determined for humans from the animal dosages utilizing the equivalent dosage methodology presented previously. As described in the section on pulmonary uptake and distribution, DCM is readily absorbed into the body following inhalation and it equilibrates rapidly across the alveolar epithelium. Therefore, the CAG considers it a virtually completely absorbed gas especially at low doses and determines equivalent human exposure as explained under Case 1 of the inhalation section. As presented in Table 5-36, the nominal exposures are nearly 15 times the human lifetime equivalent exposures. This difference, 24/6 x 7/5 = 5.6, is partly due to the use of a continuous equivalent dosage. There is an additional factor of about 2.6, however, which is attributable to the nature of the method used for determining human equivalent dosages for inhalation studies. Put another way, if DCM had been determined to be a partially soluble vapor, the unit risk slope would be lower by a factor of about 2.6.

When the incidence data given above were fitted with the continuous human equivalent exposures, the linearized multistage model yielded the following value for the 95 percent upper limit of risk:

$$q_1^* = 1.8 \times 10^{-7} (\mu g/m^3)^{-1}$$

5.3.3.3.2.2 Unit risk (mg/kg/day) and ($\mu g/l$) for oral studies. This unit risk estimate should be used only under the assumption that DCM is a potential human carcinogen. As discussed in the qualitative section, there is only limited animal evidence and no human evidence to support that assumption.

TABLE 5-36. INCIDENCE RATES OF SALIVARY GLAND REGION SARCOMAS IN MALE SPRAGUE-DAWLEY RATS IN THE DOW CHEMICAL COMPANY (1980) DICHLOROMETHANE INHALATION STUDY

Continuous human equivalent (animal nominal) exposures		Incidence rates
ppm and	$\mu g/m^3$	Number of rats with tumors/ total rats examined (%)
0 (0)	0 (0)	1/93 (1%)
34 (500)	1.2×10^5 (1.8×10^6)	0/94 (0%)
103 (1,500)	3.6×10^5 (5.2×10^6)	5/91 (5%)
240 (3,500)	8.4×10^5 (1.2×10^7)	11/88 (12%)

$$* \ 1 \text{ ppm} \times 1.2 \times 10^3 \ \mu g/m^3 \times \frac{84.9}{28.8} = 3.5 \times 10^3 \ \mu g/m^3$$

An exposure of 500 ppm DCM in air expressed as $\mu g/m^3$ is

$$500 \text{ ppm} \times \frac{3.5 \times 10^3 \ \mu g/m^3}{\text{ppm}} = 1.8 \times 10^6 \ \mu g/m^3$$

Since animal exposure was for 6 hr/day, 5 days/week, the animal continuous lifetime exposure equivalent was

$$1.8 \times 10^6 \ \mu g/m^3 \times \frac{6}{24} \times \frac{5}{7} = 3.2 \times 10^5 \ \mu g/m^3$$

The human equivalent dose for DCM is calculated first by determining the amount actually breathed by the rats. As presented in the inhalation dose equivalence section, 350 g rats breathe

$$I = 0.105 \ (0.350/0.113)^{2/3} = 0.223 \ m^3 \text{ (air)/day.}$$

Thus continuous animal dose was $3.2 \times 10^5 \ \mu g/m^3 \times 0.223 \ m^3/\text{day} = 7.15 \times 10^4 \ \mu g/\text{day}$, or, for a 350 g rat, $7.15 \times 10^4 \ \mu g/\text{day}/0.350 \text{ kg} = 2.0 \times 10^5 \ \mu g/kg/\text{day}$. The human equivalent dose is

$$2.0 \times 10^5 \ \mu g/kg/\text{day} \div (70/0.350)^{1/3} = 3.5 \times 10^4 \ \mu g/kg/\text{day}$$

or

$$3.5 \times 10^4 \ \mu g/kg/\text{day} \times \frac{70 \text{ kg}}{20 \ m^3} = 1.2 \times 10^5 \ \mu g/m^3.$$

Since publication of a final NTP report on gavage studies for rats and mice on DCM has been cancelled, there is no suitable oral study from which to estimate a unit risk.

5.3.3.3.2.3 Comparison of animal and human inhalation studies. The study of Kodak employees, yielding negative cancer results, is compared with the positive tumor results of the rat inhalation study (Dow Chemical Company, 1980). In the latter study, the salivary gland region tumors in male Sprague-Dawley rats led to a 95% upper-limit slope estimate of $q_1^* = 1.8 \times 10^{-7}$ $(\mu g/m^3)^{-1}$. If this slope factor is applied to the human inhalation study, in which time-weighted average exposure was estimated as ranging from approximately 30 to 120 ppm, the expected impact of exposure can be estimated. For this purpose, 1 $\mu g/m^3$ of DCM is equivalent to 2.9×10^{-4} ppm. Thus, the upper-limit slope in ppm is

$$q_1^* = 1.8 \times 10^{-7}\ (\mu g/m^3)^{-1} \times \frac{3.45 \times 10^3\ \mu g/m^3}{\text{ppm}} = 6.2 \times 10^{-4}\ \text{ppm}^{-1}.$$

Since this upper-limit slope is based on continuous lifetime equivalent exposure, the exposure range of 30 to 120 ppm time-weighted average must be adjusted to lifetime equivalence as follows:

$$30 \text{ ppm} \times \frac{20}{70} \text{ years} \times \frac{240}{365} \text{ days} \times \frac{8}{24} \text{ hr} = 1.88 \text{ ppm}$$

and 7.52 ppm lifetime equivalence for the 120 ppm exposure group, assuming 20 years of exposure for the 252 long-term exposure workers. Based on this upper-limit slope factor and the range of exposure, the group of 252 workers could expect an additional lifetime risk of between

$$R = 6.2 \times 10^{-4} \text{ ppm}^{-1} \times 1.88 \text{ ppm} = 1.2 \times 10^{-3} \text{ and } 4.7 \times 10^{-3}.$$

For these 252 workers, this would translate to an upper limit of between $252 \times 1.2 \times 10^{-3} = 0.3$ and 1.2 excess lifetime cancer deaths. Based on the total expected Kodak employee deaths of 65.9 (Table 5-33) for this 20-yr minimally exposed cohort, we would expect 26% of the cohort to die in the 17-yr follow-up period. Transposing this 26% to the expected excess lifetime cancer deaths, an upper limit of between 0.1 and 0.3 excess cancer deaths can be expected during the 17-yr follow-up period. The power to detect this increase from 17.77 to 18.1 cancer deaths is less than 10%. If these excess cancer deaths were from cancers of one specific site, the power would be greater, but not great enough to declare this a negative study. Even for a rare cancer, such as a liver cancer, the expected number of cases would be much less than 1. Since only deaths can be observed, the power to observe one death from liver cancer in this cohort is quite small.

Based on the analysis presented above, the study of Kodak workers exposed to DCM, showing no increase in cancer, cannot be judged as having negative results because of its low power, which is related to low exposure from a weak animal carcinogen.

5.3.3.3.2.4 Relative potency. One use of the unit risk concept is to compare the relative potencies of carcinogens. To estimate relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

Figure 5-1 is a histogram representing the frequency distribution of the potency indices of 53 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 5-37. Where human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used, in that order. Animal oral studies are selected over animal inhalation studies because most of the chemicals have been subjected to animal oral studies; this allows potency comparisons by route.

The potency index for DCM, based on salivary gland region tumors in male Sprague-Dawley rats in the Dow inhalation study (Dow Chemical Company, 1980), is 5.3×10^{-2} $(\text{mmol/kg/day})^{-1}$. This is derived as follows: The slope estimate from the Dow study, 1.8×10^{-7} $(\mu g/m^3)^{-1}$, in converted units of 6.3×10^{-4} $(\text{mg/kg/day})^{-1}$, is multiplied by the molecular weight of 84.9 to give a potency index of 5.3×10^{-2}. Rounding off of the nearest order of magnitude gives a value of 10^{-1}, which is the scale presented on the horizontal axis of Figure 5-1. The index of 5.3×10^{-2} is the least potent of the 53 suspected carcinogens. Ranking of the relative potency indices is subject to the uncertainty of comparing estimates of potency of different chemicals based on different routes of exposure to different species using studies of different

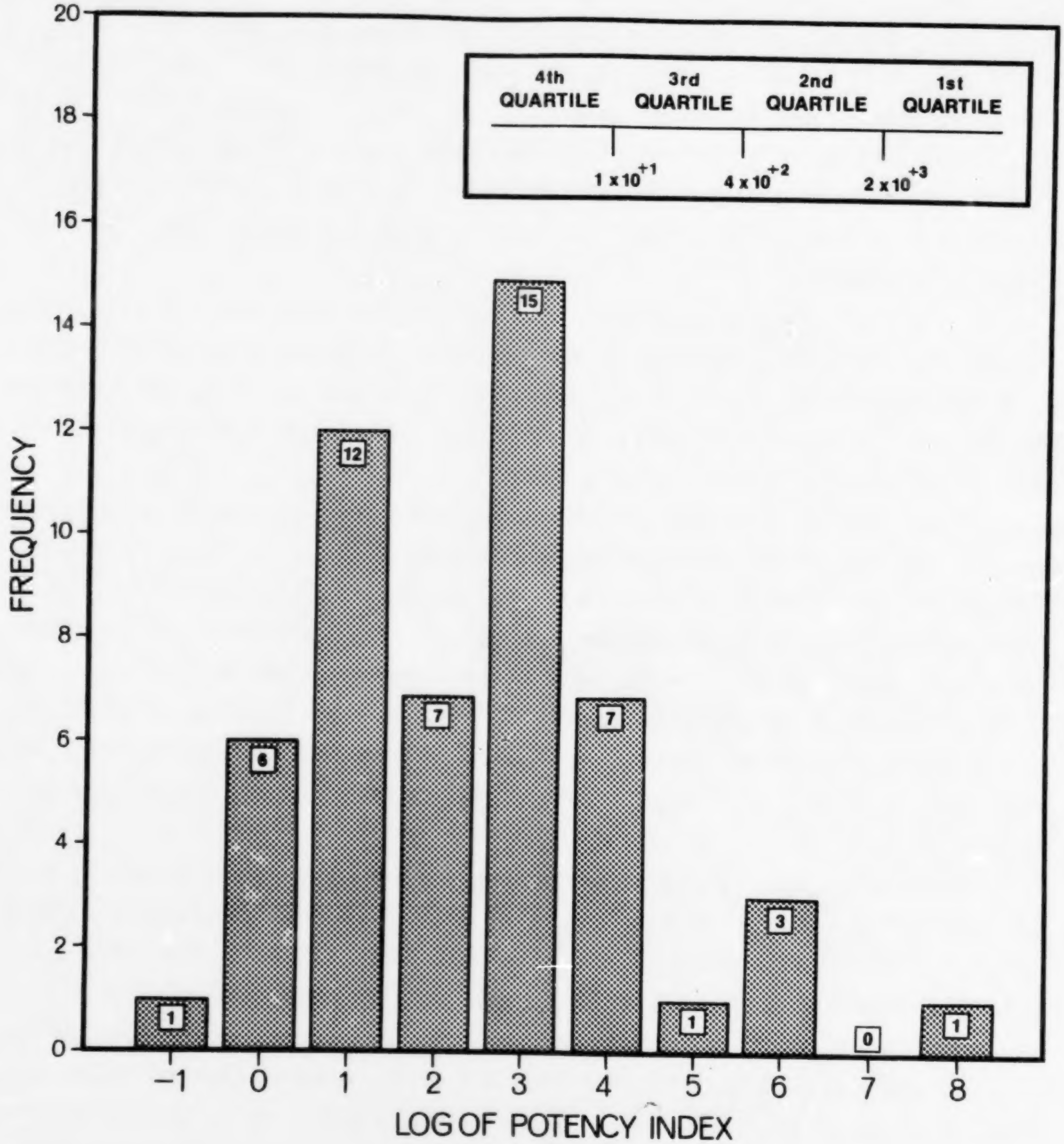

Figure 5-1. Histogram representing frequency distribution of the potency indices of 53 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 5-37. RELATIVE CARCINOGENIC POTENCIES AMONG 53 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compound	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	1162-65-8	L	S	2A	2900	312.3	$9x10^{+5}$	+6
Aldrin	309-00-2	I	L	2B	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride					$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	1	$5.2x10^{-2}$(W)	78	$4x10^{0}$	+1
Benzidene	92-87-5	S	S	1	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	7440-41-7	L	S	2A	2.6	9	$2x10^{+1}$	+1
Cadmium	7440-43-9	L	S	2A	7.8(W)	112.4	$9x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	2B	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	$7x10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

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TABLE 5-37. (continued)

Compound	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
		Humans	Animals					
Chlorinated ethanes								
1,2-dichloroethane	107-06-2	I	S	2B	$6.9x10^{-2}$	98.9	$7x10^{0}$	+1
hexachloroethane	67-72-1	I	L	3	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,2,2-tetrachloroethane	79-34-5	I	L	3	0.20	167.9	$3x10^{+1}$	+1
1,1,2-trichloroethane	79-00-5	I	L	3	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
Chloroform	67-66-3	I	S	2B	$7x10^{-2}$	119.4	$8x10^{0}$	+1
Chromium	7440-47-3	S	S	1	41(W)	100	$4x10^{+3}$	+4
DDT	50-29-3	I	S	2B	0.34	354.5	$1x10^{+2}$	+2
Dichlorobenzidine	91-94-1	I	S	2B	1.69	253.1	$4x10^{+2}$	+3
1,1-dichloroethylene	75-35-4	I	L	3	$1.47x10^{-1}$(I)	97	$1x10^{+1}$	+1
Dieldrin	60-57-1	I	S	2B	30.4	380.9	$1x10^{+4}$	+4
2,4-dinitrotoluene	121-14-2	I	S	2B	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	122-66-7	I	S	2B	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	106-89-8	I	S	2B	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	111-44-4	I	S	2B	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	542-88-1	S	S	1	9300(I)	115	$1x10^{+6}$	+6

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

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TABLE 5-37. (continued)

Compound	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	$8x10^{+3}$	+4
Ethylene oxide	75-21-8	L	L	2A	1.26(I)	44.1	$6x10^{+1}$	+2
Heptachlor	76-44-8	I	S	2B	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	3	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					4.75	290.9	$1x10^{+3}$	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	$3x10^{+3}$	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	58-89-9	I	L	2B	1.33	290.9	$4x10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	$6.2x10^{+3}$	391	$2x10^{+6}$	+6
Methylene chloride	75-09-2	I	L	3	$6.3x10^{-4}$(I)	84.9	$5x10^{-2}$	-1
Nickel	7440-02-0	L	S	2A	1.15(W)	58.7	$7x10^{+1}$	+2
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	$9x10^{+2}$	+3

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

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TABLE 5-37. (continued)

Compound	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	$4x10^{+3}$	+4
N-nitroso-N-methylurea	684-93-5	I	S	2B	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	2B	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	1336-36-3	I	S	2B	4.34	324	$1x10^{+3}$	+3
Phenols 2,4,6-trichlorophenol	88-06-2	I	S	2B	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	2B	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
Tetrachloroethylene	127-18-4	I	L	3	$3.5x10^{-2}$	165.8	$6x10^{0}$	+1
Toxaphene	8001-35-2	I	S	2B	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	79-01-6	I	L	3	$1.9x10^{-2}$	131.4	$2.5x10^{0}$	0
Vinyl chloride	75-01-4	S	S	1	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model.
2. The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.
3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

quality. Furthermore, all the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. Thus these indices are not valid for the comparison of potencies in the experimental or observational range if linearity does not exist there. The potency index for DCM, furthermore, is valid only under the assumption that DCM is a potential human carcinogen. The evidence for that is limited.

5.3.3.3.2.5 Summary of quantitative estimation. No positive epidemiologic studies exist from which to estimate a unit risk for exposure to DCM. Only one animal data set has shown increased cancers from which a unit risk assessment could be estimated. In the Dow Chemical Company (1980) rat inhalation study, there were increased sarcomas in the salivary gland region of male rats; this yielded an upper 95% limit of the potency estimate of $q_1^* = 1.8 \times 10^{-7}$ $(\mu g/m^3)^{-1}$, equivalent to $q_1^* = 6.3 \times 10^{-4}$ $(mg/kg/day)^{-1}$ by the oral route. However, no positive data exist with which to provide a direct oral route estimate. In total, there is only limited evidence that DCM is a potential human carcinogen. The unit risk estimate is valid only if one accepts that limited evidence. Further, if one chooses to express the potency of DCM relative to that of 53 chemicals evaluated as suspect carcinogens, DCM is the weakest, ranking last.

5.3.3.4 Summary. Six chronic studies of DCM administered to animals have been reported: four in rats, one in mice, and one in hamsters. The Dow Chemical Company (1980) reported the results of chronic inhalation studies in rats and hamsters. The rat study showed a small increase in the number of benign mammary tumors compared to controls in female rats at all doses and in male rats at the highest dose, as well as a statistically significant increased incidence of ventral cervical sarcomas, probably of salivary gland origin, in male rats. The response pattern of the salivary gland tumors is unusual, consisting of sarcomas only and appearing in males but not in females. In hamsters, there was an increased incidence of lymphosarcoma in females only, which was not statistically significant after correction for survival. The results of the Dow Chemical Company (1980) study have since been published by Burek et al. (1984). The second inhalation study in rats by the Dow Chemical Company (1982) reported that there were no compound-related increased incidences of any tumor type, but that the highest dose was far below that of the previous Dow inhalation study in rats. The National Coffee Association (1982a,b) drinking water study in Fischer 344 rats reported that the incidence of neoplastic nodules and/or hepatocellular carcinomas in female rats was increased significantly with respect to matched controls, but their incidence was within the range of historical control values at that laboratory. The National Coffee Association (1983) drinking water study in B6C3F1 mice also showed a borderline response of combined neoplastic nodules and hepatocellular carcinomas. The National Toxicology Program (1982) draft gavage study on rats and mice will not be published as a final report due to data discrepancies. Selected information from the gavage studies may be incorporated into the future NTP inhalation bioassay, pending the results of the in-depth audit.

There are some other inadequate animal studies in the literature. One study (Theiss et al., 1977) reported a marginally positive pulmonary adenoma response in strain A mice injected intraperitoneally with DCM. Two negative animal inhalation studies were inadequate because they were not carried out for a full lifetime (Heppel et al., 1944; MacEwen et al., 1972).

One carcinogenicity study of DCM in animals is currently in progress: an NTP 2-year bioassay by inhalation in Fischer 344/N rats and B6C3F1 mice.

Positive results in a rat embryo cell transformation study were reported by Price et al. (1978). The significance of their findings with regard to carcinogenicity is not well understood at the present time.

The epidemiologic data consist of two studies: Friedlander et al. (1978), updated by Hearne and Friedlander (1981), and Ott et al. (1983 a,b,c,d,e). Although neither study showed excessive risk, both showed sufficient deficiencies to prevent them from being judged negative studies. The Friedlander et al. study (1978) lacked great enough exposure (based on animal cancer potency estimates) to provide sufficient statistical power to detect a potential carcinogenic effect. The Ott et al. study (1983 a,b,c,d,e), among other deficiencies, lacked a sufficient latency period for site-specific cancer.

Only one animal data set has shown increased cancers from which a unit risk assessment could be estimated. In the Dow Chemical Company (1980) rat inhalation study, there were increased sarcomas in the salivary gland region of male rats; this yielded an upper 95 percent limit of the potency estimate of $q_1^* = 1.8 \times 10^{-7}$ $(\mu g/m^3)^{-1}$, equivalent to $q_1^* = 6.3 \times 10^{-4}$ $(mg/kg/day)^{-1}$ by the oral route. This upper bound unit risk estimate should be used only under the assumption that DCM is a potential human carcinogen. As discussed previously, there is only limited animal evidence and no human evidence to support that assumption.

5.3.3.5 Conclusions. Animal studies show a statistically positive salivary gland sarcoma response in male rats (Dow Chemical Company, 1980) and a borderline hepatocellular neoplastic nodule response in the rat (National Coffee Association, 1982). There is also evidence that DCM is weakly mutagenic. According to the criteria of the International Agency for Research on Cancer (IARC), the weight of evidence for carcinogenicity in animals is limited.

There was an absence of epidemiologic evidence for the carcinogenicity of DCM in a well-conducted epidemiologic study having long-term exposure. However, on the basis of animal data, the level of exposure to the individuals in the study was too low to produce an observable increase in cancer. The overall evaluation of DCM, based on IARC criteria, is Group 3, meaning that the chemical cannot be classified as to its carcinogenicity for humans.

The upper-limit unit risk for DCM, based on a rat inhalation study, is estimated to be roughly 1.8×10^{-7} for a lifetime exposure to 1 $\mu g/m^3$ in air. The above is true only under the assumption that DCM is a potential human carcinogen, although the likelihood of this is highly uncertain given the currently assessed data base. Even under that assumption, the potency of DCM is the lowest of the 53 chemicals that the CAG has evaluated as suspect carcinogens. The Environmental Health Committee of EPA's Science Advisory Board (SAB) indicated that the unit risk estimation was "scientifically" unsupportable because of the questionable value of the animal study upon which the unit risk estimation was based, and that consideration should be given to deleting the analysis from this document. The SAB noted, however, that the unit risk estimates may be useful for risk management purposes. In light of the potential need for risk management analysis, the risk estimation section has been retained.

Preliminary results of a new NTP study in mice indicate that DCM induces a high incidence of lung and liver tumors in both sexes. When the study is completed and available, the results will be evaluated, and the qualitative and quantitative portions of this assessment updated. For this reason the present report is regarded as interim.

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APPENDIX

COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimates of unit risk based on animal studies presented in the body of this document are all calculated by the use of the linearized multistage model. The reasons for its use have been detailed herein. Essentially, this model is part of a methodology that estimates a conservative linear slope at low extrapolation doses and is consistent with the data at all dose levels of the experiment. It is a nonthreshold model which holds that the upper-limit of risk predicted by a linear extrapolation to low levels of the dose-response relationship is the most plausible upper limit for the risk.

Other models have also been used for extrapolation, and include the three nonthreshold models presented here: the one-hit, the log-Probit, and the Weibull. The one-hit model is characterized by a continuous downward curvature, but is linear at low doses. It can be considered the linear form or first stage of the multistage model because of its functional form. Because of this and its downward curvature, the one-hit model will always yield estimates of low-level risk that are at least as large as those of the multistage model. Further, whenever the data can be fitted adequately by means of the one-hit model, estimates from the two procedures will be comparable.

The other two models, the log-Probit and the Weibull, are often used to fit toxicological data in the observable range, because of their general "S" curvature. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage model.

The log-Probit model was originally proposed for use in problems of biological assay, such as the assessment of potency of toxicants and drugs, and has usually been used to estimate such values as percentile lethal dose or percentile effective dose. Its development was strictly empirical, i.e., it was observed that several log dose-response relationships followed the cumulative normal probability distribution function. In fitting the cancer bioassay data, assuming an independent background, this becomes:

$$P(D;a,b,c) = c + (1-c)\,\Phi\,(a+b\log_{10}D) \qquad a,b \leq c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standarized mean of individual tolerances, and b is an estimate of the log dose-Probit response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single noncarcinogenic cell into a carcinogenic one. It has the probability distribution function:

$$P(D;a,b) = 1\text{-}\exp\text{-}(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates. The estimate a represents the background or zero dose rate, and the parameter estimated by b represents the linear component or slope of the dose-response model. In discussing the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1\text{-}\exp\text{-}(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1\text{-}\exp\text{-}(bd^k) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, this model yields low-dose estimates of risks usually significantly lower than either the multistage or one-hit models, which are linear at low doses. All three of these models usually project risk estimates significantly higher at the low exposure levels than those from the log-Probit.

The estimates of added risk for low doses for the above models are given in Table A-1 for the DCM inhalation study. Both maximum likelihood estimates and 95% upper confidence limits are presented. All estimates incorporate Abbott's correction for independent background rate.

TABLE A-1. ESTIMATES OF LOW-DOSE RISK TO HUMANS BASED ON SALIVARY GLAND REGION SARCOMAS IN MALE RATS IN THE DOW CHEMICAL COMPANY (1980) INHALATION STUDY DERIVED FROM FOUR DIFFERENT MODELS (All estimates incorporate Abbott's correction for independent background rate.)*

	Maximum likelihood estimates of additional risks				95% upper confidence limit of additional risks			
Dose $\mu g/m^3$	Multistage model	One-hit model	Weibull model	Log-Probit model	Multistage model	One-hit model	Weibull model	Log-Probit model
Males								
1 $\mu g/m^3$	2.2×10^{-8}	1.3×10^{-7}	2.6×10^{-11}	0	1.8×10^{-7}	2.0×10^{-7}	4.8×10^{-10}	3.5×10^{-31}
10 $\mu g/m^3$	2.2×10^{-7}	1.3×10^{-6}	1.1×10^{-9}	0	1.8×10^{-6}	2.0×10^{-6}	1.7×10^{-8}	1.6×10^{-22}
100 $\mu g/m^3$	2.2×10^{-6}	1.3×10^{-5}	5.0×10^{-8}	1.1×10^{-16}	1.8×10^{-5}	2.0×10^{-5}	6.1×10^{-6}	2.5×10^{-15}
1000 $\mu g/m^3$	2.2×10^{-5}	1.3×10^{-4}	2.2×10^{-6}	5.0×10^{-11}	1.8×10^{-4}	2.0×10^{-4}	2.0×10^{-5}	1.3×10^{-9}
10,000 $\mu g/m^3$	2.2×10^{-4}	1.3×10^{-3}	9.5×10^{-5}	1.7×10^{-6}	1.8×10^{-3}	2.0×10^{-3}	6.1×10^{-4}	2.4×10^{-1}

*Response fit against human equivalent dosages as presented in Table 5-36.

Human equivalent dose $\mu g/m^3$	Tumors/Total
0	1/93
1.2×10^5	0/94
3.6×10^5	5/91
8.4×10^5	11/88

*USGPO:1985-559-111-10785